File

SUMMARY OF SECOND SILAGE CONFERENCE.

Held at Beltsville, Maryland, on March 9-10, 1959

Part I

PROCEDURES AND TECHNIQUES USED IN SILAGE STUDIES

FOREWORD

Ensiling has become an important practice in the harvesting, preservation and storing of hay crops. The practice of ensiling these crops has increased because of the weather hazards, especially in the semi-humid areas where considerable loss of nutrients occur when the crop is field-cured.

Silage making practices or procedures in the past have been studied mainly by trial and error methods. It was felt that there is a definite need for more fundamental research on silage preservation and wider use of more precise methods for the evaluation of practical ensiling procedures. It was felt that further work on the evaluation of the silages produced is needed. There is also a need to standardize terminology, techniques and procedures of study as far as possible so that results from one location can be interpreted and compared directly with those at another location.

In response to these needs, the first Silage Conference was held at Beltsville, Maryland, February 20-21, 1956. At this Conference, a brief manual was developed on "Chemical and Bacteriological Procedures, Evaluation of Silages, and Techniques for Sampling and Ensiling Procedures."

Since there was considerable interest in the manual and evidence of its use by many workers, and since there had been some improvements in the methods and techniques used for conducting silage studies, it was felt an attempt should be made to revise the manual.

The Dairy Cattle Research Branch accordingly contacted the technical workers in the various states and found a considerable interest in holding a Second Conference.

A Second Conference was held at Beltsville, Maryland, March 9-10, 1959, and attended by some 50 technical workers. It should be pointed out that every effort was made to contact all those persons conducting research in a phase of silage work within reasonable distance of Beltsville for attendance at the Conference. For this purpose, we depended upon previous participants from the various states for suggestions concerning interested scientists. Any omissions which may have occurred were not intentional.

The Chairman wishes to express his thanks to the respective chairmen of each section for their excellent cooperation in developing the material for the manual.

Because it was thought that the information developed in the Conference might be useful to others than those attending the Conference, the Branch consented to have the recommendations and discussions mimeographed. In order to handle the mimeographing and binding more efficiently, the report of the Silage Conference has been divided into two parts. Part I is entitled, "Procedures and Techniques Used in Silage Studies," and Part II, "Factors Affecting Chemical and Bacteriological Changes and Factors Affecting Silage Intake and Productive Value." Individual copies can be obtained by writing to the Dairy Cattle Research Branch, Beltsville, Maryland.

L. A. Moore
Chairman

List of Research Workers Attending Conference

Allen, R. S. - Iowa
Archibald, J. G. - Massachusetts
Balestier, Elliott, Jr. - Wash., D.C.
Baxter, H. - Tennessee
Bratzler, J. W. - Pennsylvania
Briggs, R. A. - Minnesota
Cason, J. L. - New Jersey
Conley, C. - South Carolina
Craigmiles, J. P. - Georgia
Davis, R. F. - Maryland
Dickey, E. C. - Maine
Dobrogosz, W. J. - Pennsylvania
Ellzey, H. D. - Louisiana
Fenner, H. - Massachusetts
Gaunya, W. S. - Connecticut
Haenlein, G. F. W. - Delaware
Hampken, R. W. - Maryland
Hill, D. L. - Indiana
Kennedy, W. K. - New York
Lancaster, R. J. - New Zealand
Lassiter, C. A. - Michigan
Loosli, J. K. - New York
Mather, R. E. - New Jersey
McCullough, M. E. - Georgia
Meyer, T. A. - Kentucky
Miles, J. T. - Mississippi
Miller, W. J. - Georgia
Morgan, M. E. - Connecticut
Owen, J. R. - Tennessee
Ramage, C. H. - New Jersey
Richards, C. R. - Delaware
Rogers, C. F. - Ohio
Sawers, W. - Wash., D. C.
Sprague, M. A. - New Jersey
Stone, R. W. - Pennsylvania

A.R.S. Beltsville

Black, D. T.
Bouma, Cecelia
Bryant, M. P.
Campbell, L. E.
Derbyshire, J.
Gordon, C. H.
Jacobson, W. C.
Kane, E. A.
Langston, C. W.
McCalmont, J. R.
Moore, L. A.
Stafford, Barbara
Sykes, J. F.
Thomas, J. W.
Van Soest, P. J.
Waldo, D. R.
Wiseman, H. G.

Section I

CHEMICAL AND BACTERIOLOGICAL PROCEDURES

It is recognized that a need exists for expansion of fundamental research on the changes (both chemical and bacteriological) which occur during the fermentation of grasses and legumes, and mixtures of these forages, in the formation of silages. Moreover, factors which affect silage quality need further study. A recent report (USDA Tech. Bull. 1187 (1958)) has described a number of useful chemical and bacteriological procedures which should be consulted.

1. CHEMICAL PROCEDURES

a. Sample storage. It is recommended that samples be analyzed as quickly as possible. If samples must be stored, however, small quantities (400-500 g.) of silage can be refrigerated (1°-4° C.) in air-tight containers and stored for about one week with reasonable satisfaction for most chemical analyses. Mason jars or double-layer (each 1.5 mil) Saran (Dow) plastic bags are suggested. Polyethylene bags are not reliable. If samples must be stored in a frozen state, it must be recognized that some "freezing out" of moisture on the inside of the container occurs and that reincorporation of this moisture back into the sample is difficult. Prior to analysis, samples should be brought to the desired temperature in the air-tight containers. Storage of fresh forage and the product obtained during the fermentation process must be adaptable to the specific analyses to be made.

b. Dry matter determination. At the present time, there is no accurate method for measurement of the true water content in silage. It is obvious, therefore, that efforts must be directed toward development of suitable procedures for moisture determination. Until an entirely acceptable procedure is devised, it is felt that the dry matter content of silage can be estimated with reasonable satisfaction in most laboratories by either of two methods.

(1) The toluene distillation technique (AOAC, 8th Ed. p. 367 (1955)), using a 100 g. sample and Bidwell-Sterling side-arm receivers of 100 ml. capacity, is fairly rapid and sufficiently reproducible. These receivers may be ordered from Scientific Apparatus Glass Co., Bloomfield, New Jersey. This is considered by some workers to be the most reliable method for moisture determination.

(2) An oven-drying method (Parch and Tracey, "Modern Methods of Plant Analysis," Vol. I, p. 3 (1956)), which is in rather good agreement with the toluene distillation method, has been employed in some laboratories. A sample of about 1000 g. (spread out in a thin layer) is placed in a forced-air type oven at 80°C. for 2 hours, stirring after 1 hour. The temperature is then lowered to 70°C. and the sample kept

until dry (approximately 6 hours). This temperature does not remove hygroscopic water which in most cases amounts to 2.5% of the dry matter. This error helps compensate for loss in volatile organic compounds.

Since both the oven drying and toluene distillation techniques result in losses in organic matter which are accounted for as water, the need for accurate methods for true water content is acute.

The forced-air oven-drying procedure is considered adequate for determining dry matter content in fresh forages and generally is in good agreement with the toluene distillation technique. The sample size should be 1000 g., but never less than 500 g., and should be in a thin layer in a tray covered with window screen or a plastic netting (Saran is good) to prevent particle loss.

c. pH determination. It is recommended that the pH of silage be measured with the aid of calomel-glass electrode equipment. The pH measurements may be made on juice expressed from the silage, on a water-silage mixture, or in some cases made by direct contact of the silage mass with the electrodes.

d. Organic acid determination. For most laboratories, it is recommended that column chromatographic procedures be employed. A method that has been highly successful was developed by Wiseman and Irvin (J. Ag. Food Chem., 5:213 (1957)). The following modifications of the procedure have been recommended:

(1) Twenty-five g. samples are soaked overnight with an initial addition of 40 ml. of 0.6 N sulfuric acid. On the following day more 0.6 N sulfuric acid is added to the extent that 2 ml. of the liquor corresponds to 0.125 g. of dry matter.

(2) The titrant, barium hydroxide, is adjusted to 0.00500 N. From the titers of the various acids, their contents in dry matter percentage are read directly from a prepared table.

It is suggested by some workers that measurement of titratable acidity (titration of a water-silage mixture to pH 7) may be of value in a crude evaluation of silage quality.

The rapidly developing field of gas chromatography provides new procedures which may be adapted to measurements of many organic compounds in silage. The application of this technique in silage investigations is encouraged.

e. Carbohydrate fractions. For an excellent review of the carbohydrate constituents of roughages, see the report by Hansen et al., (Ill. Agr. Expt. Sta., Bull. 634, (1958)).

(1) Sugars. It is recommended that the method of Wiseman, Mallack and Jacobson (ARS-44-28, June 1958) be employed for measuring sugars in silages and forages. The following modifications of this method are suggested:

Use 0.4 g. Dowex-50W-X8 (200-400 mesh) and 1.4 g. Dowex 3 (20-50 mesh) in pure water in place of the ion-exchange resins listed in the method. These reagents are sold in their hydrogen and hydroxyl forms, thus requiring no regeneration.

Modify the Somogyi copper reagent (J. Biol. Chem., 160:61 (1945)) by doubling the prescribed amount of disodium phosphate and adding exactly 165 meq. of sodium hydroxide to two liters. With this reagent glucose and fructose give the same response.

(2) Holocellulose. The procedure of Wise et al. (Paper Trade J., 122:35 (1946)) as modified by Ely and Moore (J. Agr. Food Chem., 2:826 (1954)) is recommended.

(3) Hemicellulose. The reader is referred to methods described by Binger et al. (J. Agr. Food Chem., 2:696 (1954)) and Routley and Sullivan (J. Agr. Food Chem. 6:687 (1958)). These procedures also allow identification and quantitative estimation of the monosaccharides and uronic acids in the hemicelluloses.

(4) Fructan. It has been suggested by Percival (Brit. J. Nutr. 6:104 (1952)) that the fructans are probably the principal reserve carbohydrates used in fermentation of grasses. The analysis of forage and silage for fructans may be accomplished by techniques described by Wylam (J. Sci. Food Agric., 4:527 (1953)). Further work on the significance of this carbohydrate fraction in grass silage fermentation is needed.

(5) Hydrolyzable carbohydrate. Suitable methods (anthrone and phenol-sulfuric acid) are described in Barnett, "Silage Fermentation," Academic Press, 1954. The carbohydrates measured by these procedures include the reducing sugars and all oligosaccharides and polysaccharides that are hydrolyzed to monosaccharides by heating with 0.25 N sulfuric acid for 30 minutes.

f. Nitrogen fractions. A description of methods for determining various nitrogen fractions in fresh forage and in silages may be found in "Silage Fermentation" by Barnett (Academic Press, Inc., New York, (1954)). Some of the most important fractions to be considered in silage analysis include total nitrogen, non-protein nitrogen, soluble nitrogen, amino nitrogen, amide nitrogen, ammonia nitrogen, total volatile bases, nitrates and nitrites. Conventional procedures are applicable for measuring total, non-protein and soluble nitrogen content. In addition to Barnett's methods, some significant procedures reported by others for certain nitrogen fractions are listed as follows:

(1) Amino nitrogen. The solution remaining after removal of volatile bases may be analyzed for amino nitrogen by the gasometric ninhydrin method of Van Slyke et al. (J. Biol. Chem. 150:251 (1943)). Other methods which give acceptable values are the B-naphthoquinone-4-sulfonate method of Cagen et al. (Proc. Soc. Exptl. Biol. Med., 78:713 (1951)) and the copper phosphate method described by Sobel and associates (Proc. Soc. Exptl. Biol. Med. 95:808 (1957)).

(2) Total amide nitrogen. The distillate obtained by steam distillation of a strongly alkaline solution of non-protein nitrogen contains the volatile bases plus NH_3 resulting from the alkaline hydrolysis of glutamine and asparagine. The difference between this and the volatile base content obtained from distillation from a buffered solution (pH 8.8) represents the amide nitrogen (see Anal. Chem. 25:1528 (1953)). Kemble and Macpherson (Biochem. J. 58:46 (1954)) have outlined another method for measuring total amide and glutamine.

(3) Ammonia nitrogen. The true value for ammonia nitrogen is not always apparent by the AOAC method or by distillation in vacuo due to the possibility of contamination with other volatile bases. Kreula (Suomen Kemistilehti B. 28:94 (1955)) reports, however, that there is rather good agreement between the distillation and the Conway methods with a series of ten different silages.

(4) Total volatile bases. The method described by Watson and Ferguson (J. Agr. Sci. 27: 1 (1937)) is satisfactory. A similar procedure has been suggested by Kemble and Macpherson (Biochem. J. 58:46 (1954)).

g. Carotene. There appears to be several suitable procedures for measuring carotene content of forages and silages. It is felt that the carotene analysis is not of major importance in evaluating silage quality or the fermentation process.

2. BACTERIOLOGICAL PROCEDURES

Some suggestions and techniques for studying forage and silage bacteria are outlined on pp. 10-12 in U.S.D.A. Technical Bulletin 1187, 1958.

a. Microorganisms occurring on fresh plants. Previous work has indicated that the majority of microorganisms that occur on fresh plants are of little importance in the preservation of grass silage. Kroulik, et al. (J. Dairy Sci. 38:256-262 (1955)) showed that the microorganisms on fresh plants consisted of a heterogenous group. They were primarily aerobic, chromogenic, non-sporeforming rods; coliform bacteria, and yeasts and molds. Relatively few organisms resembling lactic acid bacteria were found. Later work by Kempton, Alan (Ph.D. - Thesis, 1958, Michigan State University, "Bacterial, biochemical and environmental interrelations in fresh and ensiled forages.") showed that the predominant bacteria on all fresh forages studied were facultative anaerobic species of Flavobacterium.

A clear understanding of the activities of the early heterogeneous flora is lacking. Many of the organisms ferment only a few sugars, produce ammonia from arginine and liquefy gelatin. Obviously, these organisms would not be too important in preserving silage (acid production), however, their role in the exhaustion of oxygen and the possibility that they might cause some changes that encourage growth of high acid producing lactic acid bacteria should not be overlooked. Furthermore, it has been found (incomplete data - DCRB) that fresh plant extracts added to culture medium favors growth of plant organisms to a much greater extent than the lactic acid bacteria. It was also shown that different portions of plants extracted in various ways stimulated strains of gram negative organisms more than lactic acid bacteria (assay strains were isolated from fresh plants). These results indicate that more emphasis should be placed on the relationship between plant nutrients and the early flora change.

b. Isolation of lactate-fermenting Clostridia. The absence of a reliable method for isolating vegetative cells of Clostridia has limited our ability to obtain a true count of these microorganisms in grass silage. A relative count may be obtained by heat shocking (80°C.- 10 minutes) the silage and determining the number of spores present. The limitations of spore counts are obvious and until a method is provided for determining vegetative cells, our knowledge of the activities of these organisms will be deficient. Work at Beltsville shows that an increase in counts of spores is always accompanied by an increase in butyric acid and a decrease in lactic acid. This is especially true in orchard grass silage. It is the opinion of workers at Beltsville that these organisms are responsible for butyric acid and poor quality silages. Stone et al. (Penna. Agr. Expt. Sta. Bull. 444 (1943)) suggest that the disappearance of lactic acid is a result of the lactic acid organism itself attacking the lactic acid converting it to acetic acid. They found only traces of butyric acid and principally lactic acid bacteria in alfalfa silages.

Several media were tried in a search for a method to culture spore-forming anaerobes. The most promising is Trypticase (BBL) medium well buffered with low sugar content. Spore counts as high as 10^7 have been obtained and in a few instances vegetative cells of Clostridia have been isolated from 10^7 dilution tubes of silage that had not been previously heat shocked.

The rolled tube technique used to culture Clostridia is the one described by Hungate (Bact. Rev. 14:1 (1950)) modified by Bryant and Burkey (J. Dairy Sci. 34:205 (1953)). Workers at Beltsville find that this is one of the simplest and most reliable methods for obtaining an anaerobic environment. The medium used for making the "total anaerobic" count is described in U.S.D.A. Technical Bulletin 1187.

We must continue our search for a method to obtain actual counts of vegetative cells of spore-forming anaerobes. Until this is accomplished, we cannot grasp the true significant of these organisms or their actual role in the fermentation process. Work is now in progress at Indiana University on this problem.

c. Isolation and characterization of lactic acid bacteria. In most silages, the lactic acid bacteria predominate but relatively little has been done on the characterization of these microorganisms. Their activity the first few hours after the forage crop has been put into the silo undoubtedly determines the type of fermentation that will result. In some silages, the pH never drops below 5, in others the pH may reach 4.2 or lower in a short period of time. There are many environmental factors that may enter the picture such as available carbohydrates, amount of packing, temperature, etc. Confusion results, however, in that silages with adequate sugar and proper environmental conditions may result in poor quality silage.

d. Recommendations.

(1) Investigators beginning work in silage bacteriology should delete detail plate counts of bacteria and enter into more fundamental aspects of the fermentation process. Making counts of bacteria is a slow, laborious process. Several workers have conducted these studies (workers at Beltsville, Stone at Pennsylvania, San Clemente at Michigan State, etc.) and the results compare favorably.

(2) Greater emphasis should be placed on the early stages of the fermentation process. These studies should include interrelations among the bacteria and the effect of plant nutrients on growth and occurrence of bacteria. We know that approximately the same numbers of lactic acid bacteria appear in both good and poor forages and they appear to be similar; however, in the poorer quality forages the lactics do not function properly (rapid drop in pH) and lactate fermenting and putrefactive anaerobes enter into the picture.

(3) Study the controlling mechanism for a sequence of bacteria in the early fermentation process (the entire first week). Gram negative rods usually appear first, then the cocci, followed by the high acid producing lactobacilli. This sequence or pattern does not always hold and it would be interesting to find out what controls this phenomenon. A considerable amount of overlapping has been observed among the morphological and physiological characteristics of organisms in silage. Weakened strains of certain species and organisms that are intermediate between certain groups have been observed. Whether this is genetic overlapping, adaptation or mutation is not known. A nutritional study of these organisms might be an approach to the problem.

R. S. Allen

Section II

EVALUATION OF SILAGES

The purpose of this section is to outline those procedures found most useful for evaluating individual samples of silage and for measuring the value of various types of silages, both by chemical techniques and by animal responses. In accurately estimating the value of any sample of silage, it would be essential to know the properties of the forage from which the silage was made, such as stage of maturity, botanical composition, crop and field history and leaf-to-stem or grain-to-stalk ratio. In any planned experiment complete and accurate records should be kept.

FARMER SILAGE SAMPLES

We are often called upon to examine samples of silage submitted by farmers. The following techniques are suggested for this purpose in the probable order of desirability:

1. pH
2. Titratable acidity
3. Moisture, protein, crude fiber and possibly the other determinations of the proximate analysis.

These determinations will provide useful guides for recommending feeding practices to accompany the presently available silage information and for improving the silage quality in the future if appropriate.

RESEARCH

In research we may be interested in efficiency of nutrient preservation, production of high quality silage and/or the value of the silage as animal feed.

PRESERVATION EFFICIENCY

The determination of preservation efficiency should be based on the comparison of the values obtained from the fresh forage arriving at the silo, plus additive, and the fermented silage, together with quantitative determinations of total material ensiled and taken from the silo. For this purpose, it appears that the following analyses would be most appropriate:

1. Dry matter
2. Sugars
3. Other carbohydrates
4. Organic acids
5. Nitrogen fractions
6. Lignin and/or methoxyl
7. Carotene

DEFINING QUALITY

The chemical determinations most useful for defining the changes and quality of the silage during and after fermentation are:

1. pH
2. Organic acids (butyric, propionic, acetic, formic, succinic and lactic acids) or titratable acidity.
3. Nitrogen fractions
4. Sugars
5. Other carbohydrates

FEEDING VALUE

The aspects of the feeding value of the silage which should be covered are digestion studies, palability and production trials.

Digestibility. In experiments designed to determine the digestibility or feeding value of silages, the following determinations are suggested:

1. Energy plus protein or proximate analyses
2. Lignin and/or methoxyl
3. Cellulose and/or lignin
4. Carotene

In digestion studies, the silage may be the only feed except when protein or other specific deficiency might result. Further studies should be made comparing the value of different species of animals for determining digestibility of silages. Studies should also be made to determine whether digestibility by high producing cows is the same as for low producing or dry cows, as well as the possible interrelationships of level of concentrate feeding and digestibility of silage with high producing cows. Standard digestion trial procedures are applicable to silages as to other rations.

Palatability. Technically, palatability implies choice, but the measurement of greater value is the total intake when given no choice. Trials to compare total intake of different silages should be set up to equalize carryover effects, such as by a balanced pair of 3x3 latin squares, with replication. Transition periods should be of at least seven days duration with experimental periods of seven days minimum duration. Because of the lack of information, specific comparisons should be made between the results of free choice palatability trials (with adequate control of position and carryover effects) and total intake trials on the same silages. Under some circumstances, it may be more desirable to run continuous trials of two or three months duration or longer to determine the long time acceptability of the silage.

Milk production and growth. For evaluation of milk producing or growth potential, two phases might be considered, first, the protein and energy value of the silage itself, and, secondly, any special

properties which the silage might have. Such special properties might be demonstrated with relatively small amounts of silage in the total ration, or might not be demonstrated unless silage made up the major part of the ration.

Reversal designs and continuous designs may both be used for either growth or milk production studies. The experimental periods in reversal studies should be a minimum of four weeks for milk production trials and a minimum of eight weeks for growth trials. Longer time trials are to be preferred, and continuous trials should supplement the reversal experiments.

Reversal designs have advantages over continuous trials with respect to balance and elimination of the influence of animal differences on the comparison in question. However, with the variability demonstrated by many silages and the relatively slow adjustment of some animals to changes in silage, continuous trials also have advantages. These may be made more sensitive by the elimination of a large part of the effect of animal differences by covariance adjustment for production or rate of growth in preliminary periods.

In heifer growth studies, the silage should be the sole feed except as supplements are necessary to prevent deficiencies. It is suggested that when used in milk production studies, the silage being tested should make up the largest possible proportion of the total ration for most accurate evaluation. Cows of high milk-producing potential and cows in the early part of the lactation are the most sensitive indicators of differences in feed quality. For such cows, since concentrate may be needed, in limited amounts, the equalized feeding procedure of Lucas (J. Dairy Sci. 26:1011. 1943) is the most desirable system of adjusting concentrate allowance.

It has been demonstrated that the most accurate method of estimating gains or losses in body weight is to take the initial weight after the animals have become adjusted to the experimental ration because of the large changes in relative amount of fill.

J. K. Loosli
R. E. Mather

Section III

ENSILING PROCEDURES

The committee recognized that adequate studies on sampling and ensiling procedures were lacking, therefore, recommendations were based upon the limited research data available and the experience and observations of the workers present. The committee attempted to set minimum standards for sampling silage and to recommend desireble standards for ensiling procedures. Most of the recommendations were made on the premise that losses of dry matter and other nutrients during the ensiling period were being measured. Obviously, sampling procedures for characterizing the quality of a silage do not have to be as detailed or as exact as when loss measurements are being made.

SAMPLING AT FILLING AND EMPTYING TIME

a. Large silos (farm size). When input-output data for dry matter and other nutrients are being gathered, at least one sample should be taken from each load of forage. Grab samples should be obtained throughout the load as the forage is being removed from the wagon or truck. If an additive is spread over the top of the load care must be exercised that it is not included in the grab sample of forage. Enough grab samples should be taken that the composite sample will be 10 to 15 pounds in size. The entire sample can be oven dried, provided this does not conflict with the dry matter determination, or if necessary, it can be thoroughly mixed and a sub-sample of not less than 1000 grams can be used. The amount of additive applied to each load should be recorded and during the filling period the preservative should be sampled adequately so that its dry matter and chemical composition can be characterized. The amount of dry matter and other constituents ensiled should be determined by calculation rather than attempting to obtain representative samples after the forage and additives have been mixed.

When the silo is being emptied the silage should be sampled close to the time of weighing by taking grab samples as the cart is filled or emptied. The silage should be sampled daily and at least one composite sample of 10 to 15 pounds should be obtained for each 2 tons of silage. Precautions should be taken to avoid evaporation of moisture and fermentation of the sample from the time it is taken until the time of chemical analysis. The composite sample for each 2 tons of silage should be thoroughly mixed in a cool room and a 2-quart sub-sample taken for moisture and chemical determinations.

b. Small experimental silos (1 1/2 ton capacity or smaller)[1,2]. As the silos are filled, frequent grab samples of the forage should be taken so that the composite sample weighs from 15 pounds, for silos with 100 pound capacity, to 30 pounds for silos with 1 1/2 ton capacity. Four to eight 1000 gram sub-samples should be oven dried to determine the moisture content of the ensiled forage. (If adequate oven space is lacking, the size of the sub-samples can be reduced to 500 grams). The forage and additives should be weighed and sampled separately as recommended for farm size silos.

Whenever possible, all of the silage should be removed from the experimental silos at one time, thoroughly mixed and grab samples totaling 15 to 30 pounds obtained throughout the pile. Four to eight sub-samples should be used for the moisture determination as outlined in Section I. The sub-samples for chemical determinations should be at least two in number and one quart in size. If the silage is to be fed to animals it can be weighed into individual daily rations, frozen and stored.

If the silage is to be fed over a 10 to 14 day period from the silo, a suitable grab sample for the moisture determination should be taken daily as the weighing cart is being filled or emptied. A smaller grab sample also should be taken daily and held under refrigeration until the silo is emptied. Then all samples can be composited, mixed, and two one-quart sub-samples can be taken for chemical determinations.

Special precautions must be taken to avoid loss of moisture and other volatile materials from the forage and silage samples. All samples should be mixed and handled as rapidly as possible in cool rooms.

It must be recognized that while detailed sampling of the silage in the individual silo will yield a more accurate estimate of the moisture content and chemical composition of the silage it does not provide a satisfactory error variance for comparing two or more treatments. A valid error term can only be obtained by having two or more silos (replications) for each treatment. Adequate replication can be obtained by having several silos of each treatment in each experiment and/or by repeating the same experiment, a sufficient number of times to provide the necessary replication. If the latter method of replication is used the individual treatments should be reassigned at random to the individual silos each time the experiment is repeated.

---

[1]Kennedy, W. K. Laboratory equipment for silage studies. Agronomy Journal, Vol. 48:262-264. 1956.

[2]Allred, K. R. and W. K. Kennedy. The use of small silos to determine dry matter losses during ensiling. Agronomy Journal, Vol. 48:308-313. 1956.

SAMPLING DURING STORAGE (FARM SIZE AND EXPERIMENTAL SILOS)

a. Technic in sampling from portholes. A representative sample of the silage in small silos can be obtained from a porthole by means of a core sampler that is inserted horizontally half way through the silo. The hole left by the core sampler does not need to be filled but the seal on the porthole must be air-tight. Core samples taken from farm sized silos do not yield dry matter, chemical or bacteriological values that are representative of the whole silo. They cannot be well identified with the stored forage because of variability from load to load and vertical settling of the silage.

The design of the core sampler is not too important if the silage is to be sampled for dry matter, chemical and/or biological determinations. If bulk density measurements are desired, the cutting edge of the core sampler must be kept very sharp and silage must not be allowed to accumulate near the cutting edge so that it is forced ahead of the tube. Complete details of a core sampler suitable for bulk density measurements are available from C. K. Otis, Department of Agricultural Engineering, University of Minnesota.

b. Frequency of sampling. Only one sample can be removed from a given porthole if bulk density measurements are made. If the samples are to be used only for dry matter, chemical and/or biological measureemnts, several borings can be made from one porthole by varying the angle of the core sampler. No particular disadvantages are seen to taking samples at different times from the same porthole if the porthole is tightly sealed between borings and as long as the sample can be taken from undisturbed silage.

FILLING TECHNIQUE FOR VERTICAL SILOS

a. Simultaneous filling of several silos with comparable forage. If comparisons between ensiling methods, such as the addition of different preservatives, are to be made using two or more large silos, the silos should be filled with alternate loads of forage from the same field or fields. To be representative of farm conditions, the silos should be filled as rapidly as possible and a minimum of 10 feet of new silage should be added daily to each silo of 8 to 12 feet in diameter. To assure reasonably rapid filling at least one chopper should be used for each two silos. In order to avoid variability in the quality of silage within the silo which might affect a feeding experiment as well as adequate sampling for the measurement of losses within the silo due to continued exposure of the silage surface to air, the filling operation should be continuous and not discontinued over the weekend. When an interruption in filling is necessary, the experimental silage should be sealed off and the seal removed before refilling begins.

b. Weighing. All loads of forage should be weighed just prior to unloading. Tare weights on vehicles should be determined every three hours and during inclement weather, when mud and moisture may accumulate on the truck or wagon, more frequent tare weights should be made.

Scales used for weighing the forage at the time of filling and the silage at the time of feeding should be checked by the official weights and standards at least once a year. The outdoor scales should be checked just prior to the ensiling period.

c. Application of additives. Additives should be added uniformly at the blower. Chemical additives should be mechanically metered with close supervision onto the forage as it enters the blower or elevator. Distribution of the feed additives can be made on top of the load or at the blower, but if spread on top of the load care must be used when sampling the forage so as not to include any of the additive.

d. Distribution and tramping in silo. Continuous distribution (manually or mechanically when possible) is recommended. If the forage is distributed uniformly against the walls of the silo and allowed to roll towards the center by the force of gravity, compaction will be the greatest at the outer edges of the silo and lowest in the center. This will allow the juice to drain to the center and out the drain in the bottom of the silo. Several workers have reported that this method of distributing the silage simplifies the collection of seepage losses.

Uniform distribution throughout the silo also is satisfactory but seepage flows to the walls and then down the silo. Considerable juice may flow between the staves and from around the doors of silos that are not extremely tight. This increases the collection problem if seepage losses are to be measured.

e. Top seal. Whenever possible, a moisture-proof covering should be placed over the ensiled forage followed by additional forage, wet sawdust or other material which will hold the seal tight against the forage and prevent the entrance or movement of air. If the moisture-proof covering is not used, a layer of burlap, roofing felt, reinforced paper or similar material should be spread over the forage to permit the addition of about 2 feet of forage prior to the completion of silo filling. Top spoilage can be determined as outlined in this Section under Surface losses.

f. Seepage collection. Precautions must be taken to intercept all the seepage and to measure the juice by total collection or continuous metering. Daily sampling of the juice for chemical analysis is desirable. If the individual daily samples are not analysed they may be composited by using aliquots proportional to the total daily juice flow. Samples can be stored by freezing or in a refrigerator with toluene as a preservative.

FILLING TECHNIQUE FOR HORIZONTAL SILOS

a. Simultaneous filling of several silos with comparable forage and weighing of the forage. The methods recommended for vertical silos above should be followed for horizontal silos. Filling of the silos should be as rapid as possible from one end and a minimum depth of three feet of compacted silage in the area of the silo being filled should be added daily.

b. Application of additive. An additive may be applied by spreading it by hand over each load of forage after the herbage has been distributed in the silo. When chemical additives are being used they may be applied to the forage at the time of cutting by means of an applicator on the field chopper if the topography is level to gently rolling and the fields are 10 or more acres in size. It is not practical to apply additives on hilly, rough or small fields as the chopper cannot be operated at a uniform speed.

c. Distributing and packing. Each load of forage should be properly distributed and then packed with a tractor. Good packing is of utmost importance in horizontal silos and the investigator must be sure that the forage is being packed adequately. For relatively small horizontal silos, wheel tractors are better for packing forage than crawler tractors. Dry or mature forage may require more packing than high moisture or immature forage.

d. Sealing procedures. It is desirable to seal the exposed surfaces of a horizontal silo as soon as filling and packing is completed. Moisture-proof plastic films appear to offer the most promise for covering horizontal silos. Such films should be carefully spread over the forage and then covered with sawdust, limestone, or other material which will hold them firmly against the silage. The edges of the plastic film should be buried or heavily weighted so that air cannot enter from the sides. The silo should be so constructed and covered that precipitation will be shed by the cover and that ground and surface water will be intercepted and diverted away from the silo.

If no top seal is used, the top of the silo should be repacked with a tractor three or four times during the two-weeks following filling. This repacking will mash the spoiled silage so that it will form a seal and be less permeable to air and water.

e. Seepage. Seepage losses can be measured by trapping all the juice flowing from the horizontal silo and then using the same techniques described in Seepage collection. Dilution of the seepage by rain or ground water must be prevented by proper ditching and a roof or moisture-proof top seal.

FILLING TECHNIQUE IN SMALL EXPERIMENTAL SILOS

a. Replication. The number of replications depend upon the measurements that are to be made. The variability of some measurements is much greater than the variability of others. Uniformity trials to determine the variability between silos are needed and until such data are available, recommendations cannot be made. In all small silo experimentation such factors as pressure and temperature should be held constant or measured.

b. Simultaneous filling of several silos with comparable forage. The length of time between cutting and ensiling may have a marked influence upon the ensiling process. Thus, all silos within a replication should be filled in the shortest possible interval of time unless time of ensiling is a treatment. The design of the experiment should be such that any difference in the time between cutting and ensiling the forage does not bias the results.

c. Method of mixing additives. In experimental silos with a capacity of several hundred pounds up to 1 1/2 tons, the additive can be applied to the forage as it is being ensiled. The additive should be weighed and sampled separately from the forage as recommended under Large silos.

In very small laboratory silos, the additive can be thoroughly mixed with the forage immediately prior to ensiling.

d. Weighing. Where possible, the empty experimental silos should be weighed immediately prior to filling and immediately after filling has been completed. This technique is preferable to weighing the forage as it is ensiled since small losses of forage and errors in recording the weights of several lots of forage are likely to occur.

e. Specifications for small experimental silos. Until more data on the influence of pressure upon the ensiling process and dry matter losses are available a definite pressure should be applied to small experimental silos. In order to standardize the pressure used by different workers, the following recommendations are suggested: For silos with a capacity of several hundred pounds up to 1 1/2 tons, a weight of 80 pounds per square foot should be used. For very small laboratory silos a pressure of 5 pounds per square inch should be used. Workers are encouraged to include pressure as one of the variables in their silage research and to use the above recommendations as their standard treatment.

In all experimental silos, provisions should be made to allow the escape of gasses so that the gas surrounding and within the silage is not concentrated or under pressure.

TEMPERATURE DATA

The relationship of temperature to the ensiling process is not fully understood and more data are needed. Changes in silage temperatures can be measured by recording thermocouples or by reading the thermocouples at definite time intervals. Studies have indicated that at any given depth the lateral temperature gradient is small. If an appreciable rise in temperature occurs in the silage, the vertical temperature gradient may be large, especially near the top. It is important, therefore, that the thermocouples are located in such a manner that the vertical profile of the temperature gradient can be characterized. The thermocouples must be placed in the silo in such a way that the settling forage will not break the lead wires.

In large silos, temperature data may be recorded over much or all of the storage period but usually there is little need to continue recording the data once the temperature of the silage starts to decline to the mean daily air temperature. There is little need for recording temperature in silos of one or two ton capacity once the ambient temperature has become the dominant influence on silage temperatures.

In very small laboratory silos, the temperature of the silage is determined by the surrounding air temperature which should be recorded. Studies which include temperature as a variable are needed.

MEASURING ENSILING LOSSES

Ensiling losses can be divided into three categories: (a) surface losses (top and side spoilage), (b) seepage losses, and (c) fermentation and respiration losses (intangible losses). The measurement of each of these losses poses certain problems and suggested procedures are outlined below.

a. Surface losses. The ensiling losses due to surface spoilage are difficult to measure if the entire mass of silage must be considered. Spreading a permeable covering such as saran screening (14 meshes to the inch) or burlap over the forage prior to ensiling the last few loads will help to confine the amount of good and spoiled silage which will have to be weighed. Top spoilage above the separator should be expressed in weight of original dry matter lost per unit area rather than as a percentage of the total forage ensiled. With these data the loss can be calculated for any size silo on the basis of total weight of silage in the silo.

The damaging effect of exposure to air may not be confined solely to the observed surface spoilage. Low concentrations of air permeating into the silage may increase the so-called fermentation losses and materially alter the chemical composition of the silage without causing sufficient deterioration to be recognized as top spoilage. The buried bag technique outlined below under Fermentation and respiration will help to establish the magnitude of these losses. (Also, see Cornell Univ. Exp. Sta. Bul. 912, 1955).

b. Seepage losses. Precautions must be taken to intercept all the seepage and to measure the juice by total collection or continuous metering. Daily sampling of the juice for dry matter content and chemical analysis is desirable. If the individual daily samples are not analysed they may be composited by using aliquots proportional to the total daily juice flow. Samples can be stored by freezing or in a refrigerator with toluene as a preservative.

Dilution of the seepage from horizontal silos by rain or ground water must be prevented by proper ditching and a roof or moisture-proof top seal.

c. Fermentation and respiration (intangible)losses. Fermentation and respiration losses can be estimated from total input-output data. The total loss of dry matter is calculated, and the seepage and surface losses are subtracted. The remaining ensiling losses are attributed to fermentation and respiration. Total input-output data are difficult to obtain in large farm-sized silos and the buried bag technique is a suitable substitute. Furthermore, the use of a large number of bags allows the worker to calculate the magnitude of his sampling error and to establish confidence limits for the observed mean value. The confidence limits should be reported with the mean.

During the filling operation, a forage sample of about 3000 grams is removed from the blower stream or from the load as it is being emptied into the silo. Two 1000 gram samples are weighed from the sample. One sample is placed in a saran mesh bag (1¼ meshes to the inch), numbered, and placed in the silo. The other sample receives the same number and is dried in an oven at 70° C. to determine the dry weight. If facilities and labor permit, two bag samples should be placed in each ton of forage ensiled. It is important that the bag samples be placed in the silo in such a manner that the silage is sampled both vertically and horizontally.

When the silo is opened, the bags are collected as they are uncovered and the moisture content of the silage is determined by the approved method (see Section I). The resulting weight of dry matter is compared with the weight of the dry matter for the paired sample that was oven dried at the time of ensiling. The difference in total dry matter content of the paired samples represents fermentation and respiration losses and seepage losses. Seepage losses must be subtracted if fermenation and respiration losses are to be estimated separately.

If the approved method of moisture determination proves to be too expensive for the number of bag samples required in a large silo the relationship between the approved method and the oven dried method can be determined on 25 paired samples. The remaining bag samples can be oven dried and the values corrected by means of a regression formula.

Light muslin bags may be used in lieu of plastic mesh bags but variability between samples will be slightly higher.

Plastic mesh bags 12"x24" in size are adequate for 1000 gram samples. The bags should be stitched with nylon or dacron thread rather than cotton thread if the bags are to be placed where surface spoilage is likely to occur.

Note: Address for obtaining saran mesh is: Chicopee Mills, 37 Worth Street, New York, New York

W. K. Kennedy

SILAGE SCORECARDS

At the first Silage Conference in Febru[illegible], 1956, a committee was appointed to attempt to develop a silage scorecard. The committee was composed of agronomists, animal and dairy husbandmen and bacteriologists.

It was realized that any scoring system has its drawbacks and the first few attempts to standardize a scorecard met with numerous complications. The committee recognized the shortcomings of their attempts and contacted scientists in the silage field for suggestions and criticism.

After several revisions, a scorecard for corn and grass silage has been developed and is presently being used at various institutions. The scorecard for grass silage is shown below:

Grass Silage Scorecard
(Grasses, Legumes or Combinations of Both)

CROP QUALITY (40 points) BASED ON STAGE OF GROWTH AT CUTTING

Sample No. _____
Composition
% legume _____
% grass _____

		Possible score	Score given
1.	Before blossom or early heading (fine stems high leaf content)	36-40	_____
2.	Early blossom	31-35	_____
3.	Mid-to-late bloom	21-30	_____
4.	Seed stage (very stemmy, coarse, low leaf content)	10-20	_____

PRESERVATION (60 points) BASED ON COLOR AND ODOR

A. COLOR (30 points)

1.	DESIRABLE - Natural forage green or slightly yellowish green. Light to dark green depending on crop and/or additive used. Red clover may have a darker color	26-30	_____
2.	ACCEPTABLE - Deep dark green or very yellowish-green or slight brownish green	16-25	_____
3.	UNDESIRABLE - Brown or black indicating excessive heating or putrefaction. Predominantly white or gray indicating excessive mold	5-15	_____

Grass Silage Scorecard (Continued)

B. ODOR (30 points)

1.	DESIRABLE - Clean, pleasant with no indication of putrefaction	26-30	_____
2.	ACCEPTABLE - Somewhat strong, yeasty, fruity or musty, slight burnt odor, sweet	16-25	_____
3.	UNDESIRABLE - Strong, burnt or caramelized odor indicating excessive heating. Sliminess and a putrid odor indicate improper fermentation. Very musty or moldy odor with excessive mold visible	5-15	_____
	TOTAL SCORE	100	_____

MOISTURE CONTENT: High moisture silage (75% or above) will contain less feed value per pound than lower moisture silage. High moisture may indicate excessive juice loss with loss of nutrients. However, heavy nutrient loss may result from ensiling material too dry to pack well. Moisture content can be approximated by squeezing in the hand, if juice runs free it is high moisture.

Using the scorecard: This represents an attempt to evaluate grass silage on the basis of the feeding value of the material ensiled and the preservation of that feeding value. One small sample of silage will not necessarily be representative of an entire silo full of silage -- there is much variation within the silo, due to location in the silo, separation in the silo when filling, and variation of the material in the field. Rather, use this scorecard as a guide to further evaluate your silages as a feed. By critical evaluation you can wisely supplement your silage and possibly insure better silage next year.

QUALITY: Forages, grasses and legumes, have higher digestibility and contain more protein in the early stages of growth. Alfalfa should be harvested by early bloom stage, clovers by 1/2 bloom stage, and the grasses before flowering for highest quality and good yield in terms of milk or useful feed nutrients per acre. Late cut, mature, stemmy forage even though well preserved cannot make high quality feed. Foreign Matter: Weeds and stubble may also have a good preservation, but the total amount of nutrients per acre will be reduced due to lower quality and yields. Although not listed in this scorecard, judgment should be used in the evaluation of silages with high foreign matter content and those samples should be penalized.

COLOR: Natural rather than artificial light should be used in grading samples on color. A natural color is desired and slight changes should not be seriously penalized. Dark brown or charred black is an indication

of excessive heating. This is usually caused by poor packing or material with too low moisture content. Silage on the outside of stacks is usually this color, even where the forage was of high moisture when stacked. Molds indicate a feed loss and are a result of air. To prevent this molding, use higher moisture material, pack better, or provide a cover of plastic film, sawdust, or soil. Deep green or black is often seen in very high moisture silage. It is sometimes associated with strong odors and occasionally with a slippery, slimy feel. This last is seriously objectionable.

ODOR: Silage odors range from a very mild crushed grass smell to very strong and penetrating. Odor reflects the type of silage fermentation. While silages with strong odors often are objectionable to people who must handle them, they may still be good feed and readily eaten by livestock. These objectionable silages, however, should not be kept in storage for too long a period. They must be fed carefully to prevent "off-flavors" in milk. Strong ammonia and moldy or musty odors indicate considerable loss in feed value and should be heavily penalized. Reserve the "DESIRABLE" rating for silages with no strong, objectionable odors. High moisture silages are usually the ones with the strong odors. Addition of preservatives or careful wilting may insure a proper fermentation and a desirable odor.

SCORING: Excellent silage 90-100; good silage 76-89; fair silage 61-76; poor silage 60 and below.

It is hoped that the scorecards will be used as widely as possible and any suggestions for revision or constructive criticism will be welcomed by the committee.

PLASTIC BAG SILOS (Field Size Units)

This technique assumes that approximately 9 to 10 tons is an acceptable size for total dry matter losses. The method is versatile in many ways offering opportunity for ensiling conventional quantities of forage from field size plots, feeding reasonably small lots to experimental animals, and locating in convenient places. Reasonably uniform and consistent results have been obtained and essentially air tight conditions reduce surface spoilage problems to a minimum. This method may have merit for making broad comparisons such as between crops, crop treatments, and preservatives.

Plastic Bags. Polyvinyl chloride tubes from 6-10 feet in diameter are available from the Bakelite Company, Bound Brook, New Jersey in thicknesses of from 4 to 8 mil. These can be used with confidence at air temperatures ranging from +110° to -10°F. Pressure sensitive tapes are available with packaged units or from Permacel Tape Co., New Brunswick, New Jersey.

Location. The location should be one of level ground, as close as possible to the place of utilization, and in an elevated position to facilitate placing the seepage collection barrels below the level of the silo. This may be accomplished by bulldozing a level area on the side of the grade or by sinking the collection barrels in the ground. The area should be raked smooth of sharp sticks and stones. A paved surface is ideal but not necessary.

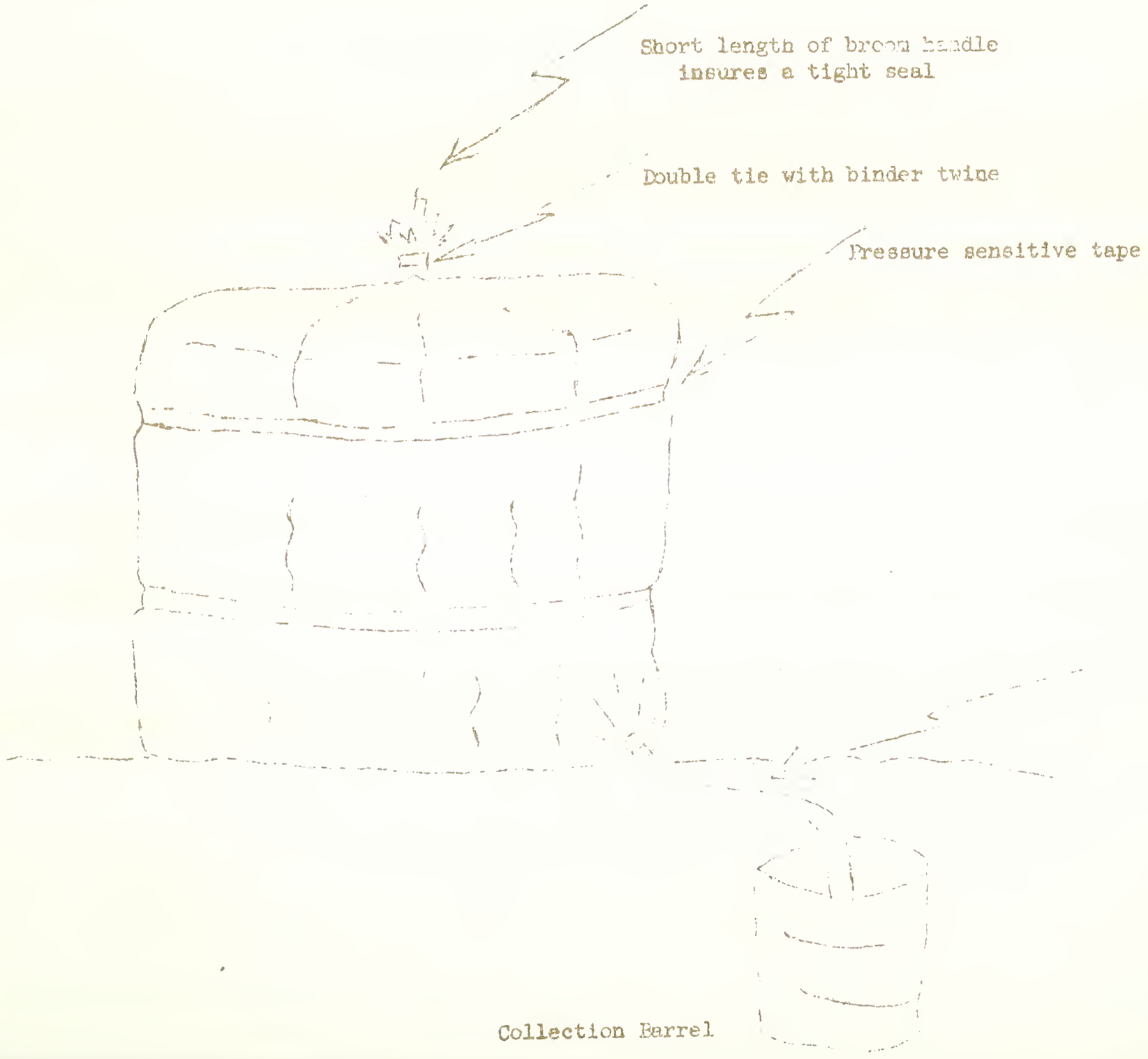
Stacking Technique. The plastic is gathered into a doughnut, placed on smooth ground, with the lower lip of the plastic extending to the center where it is gathered and tied with binder twine. A cylindrical form made of sheet metal or of snow fence material should be rested on the plastic inside and tightly against the ring of gathered film. The form should be filled as rapidly as possible with the test forage and packed well around the perimeter. Filling may continue until the height of the stack is about equal to the diameter. The form is then removed and the plastic sleeve drawn up tightly and tied securely at the top. Ten foot diameter stacks packed well and filled to 11 feet will settle to 10 feet and contain approximately 9 1/2-10 tons of grass-legume silage at 75% moisture content. Six foot diameter units filled to 6 feet will hold about 2 1/2 tons.

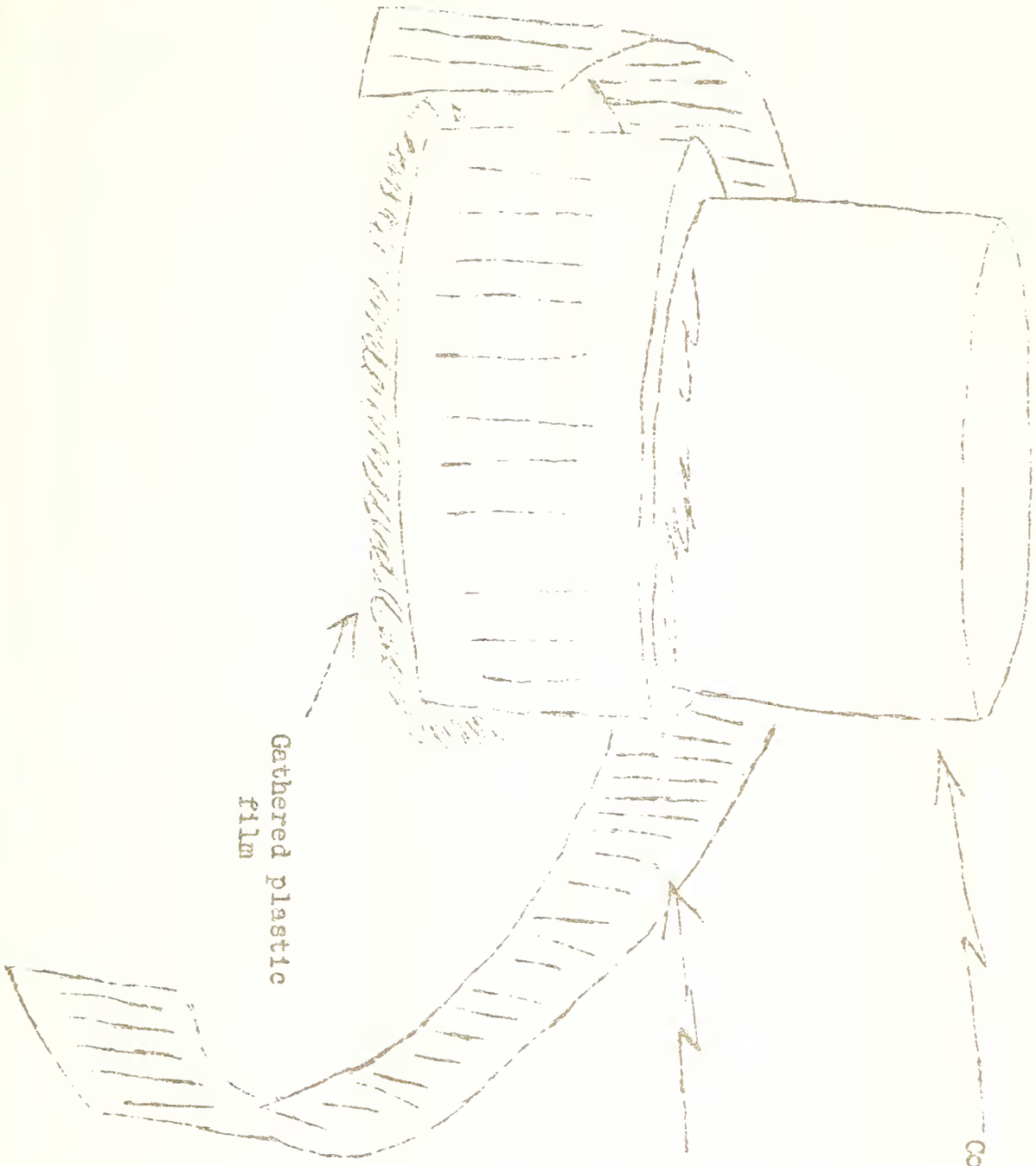
An alternate method is to form a ring of 1 x 2 inch welded wire fencing, bringing up the edges of the plastic tube inside the fence and laying over the top until the first ring is filled. The film is then temporarily pulled over the forage until a second ring is placed and tied on top of the first. The plastic is then lifted over the second form as before. After filling is complete the film is gathered tightly as before and tied securely.

Seepage Collection. After the stacked silages have had an opportunity to settle for 24 hours and seepage has been observed to collect, the lowest side of the silo should be determined. At this point, a small incision should be made permitting the entrance of 3/4 inch plastic hose after which the plastic film should be tightly tied around the hose making a tight seal and the hose led directly to the collection barrel. It may be desirable to insert this hose immediately after filling in which case a screw clamp or stopper should close the tube until seepage begins to flow.

Dry Matter Losses. Samples are taken of green and ensiled forage as described elsewhere. Forage is weighed at filling and again on opening by removing by hand or with fork lift equipment to a wagon. Differences in weight, chemical analyses, and seepage measurements establish dry matter losses and identity of the nature of losses obtained.

Diagrams of plastic bag silos follow. Further information may be obtained from M. A. Sprague, Rutgers University, New Brunswick, New Jersey.


Short length of broom handle
insures a tight seal
Double tie with binder twine
Pressure sensitive tape
Collection Barrel


Completed stack
of forage
Wire mesh or
snow fence
Gathered plastic
film

IMPROVED LABORATORY SILO EQUIPMENT DEVELOPED AT THE UNIVERSITY OF MINNESOTA FOR THE STUDY OF FACTORS AFFECTING THE PRODUCTION OF NO_2 GAS FROM CROPS DURING THE ENSILING PROCESS

Laboratory silo equipment has been constructed and evaluated for use in determining environmental and other factors affecting the silage fermentation process that contribute to the production of nitrogen dioxide gas. It consists of twenty-seven Lucite silos arranged in 3 units of nine silos each. A picture showing the interior of one of the units appears in this report. The silos, that have a capacity of 5 or 6 lbs. of chopped green crop, can be seen at the top. The pistons seen through the silo walls apply pressure to the sample through a piston rod actuated by controlled air pressure in the stell cylinders just under the silos. The pistons are designed to facilitate movement of expressed juice and gas from the sample through a tube to the juice collection bottles located between the air cylinders where the juice accumulates for weighing and the gas passes on through to vertical glass manifolds that also collect gas from the tops of the silos and conduct it to NO_2 detectors and thence to the large total gas collection bottles located on the bottom shelf of the unit. When in operation an insulated panel fits into the lower opening and transparent plexiglass panels fit into the upper opening so that readings can be taken without seriously disturbing the heating system that maintains uniform temperature within the cabinet. A black-out curtain is drawn over the entire side of the cabinet to exclude light.

The schematic diagram of one silo and its auxiliary equipment shows how it operates. Air pressure is maintained within close limits by manostats. A special grooved vent and gas collection plate above the sample is accessible through two holes in the top of the silo. Normally, one hole only is used but the other can be used for exposing the surface of the sample to atmospheres of known gas composition. When operating under air tight conditions a slight vacuum (2 or 3 inches of acidified water) is maintained on the gas collection bottle minimizing escape of lethal gases through possible leaks in the system.

The system used for maintaining air pressure on the air mains is shown in the simplified diagram at the right where one of the three air mains is shown. Air from the compressor enters at the top of a stand-pipe provided with a blow-off valve at the bottom for removing water from the line. Air is tapped from the stand-pipe and is reduced to within a few pounds per sq. in. of the pressure to be maintained. It then passes through a filter and thence to a needle valve that introduces air into the vertical section of the main. At this point, a manostat is installed that controls the pressure on the main at the desired level. A manometer also connected to the main indicates the pressure being maintained. Controlled pressure connections to the other two units are shown on the top of the unit. Also, on this diagram is shown the syphon manifold and adjustable tank that controls the liquid level for the gas bottle syphons.

Each of the three units have independent temperature control so that the effect of temperature on the fermentation process can be determined. Within each unit, silos are arranged in three groups of three. Each group of three samples can be exposed to the same pressure as the other two groups or can be subjected to any one of three pressures by manipulating valves to connect them to the proper air main. Thus, the effect of pressure on the fermentation can be studied.

Advantages of the design features used in this equipment are are as follows:

1. Only 150 lbs. of green crop are needed to fill all 27 silos thus making easier the selection of identical crop material for the tests.

2. Provision is made for three replicates of each variable being studied.

3. Controlled temperature (2° max. differential) and pressure (0.2 in. Hg differential) on silage samples.

4. Juice flow in the silage sample is similar to that in a large silo, i.e. gravity pulls it from lower density material through denser material which in these silos is next to the piston.

5. Facilities are provided for gas and juice to move freely from the sample and provisions are made for their collection and measurement.

6. Light intensity on the silage sample can be varied.

7. Volume of the sample can be read directly at any time by a cubic foot scale on each silo.

8. The color and condition of the sample can be observed through transparent walls.

9. A large part of the surface of the silage samples can be exposed to air or to a gas of any known composition if desired.

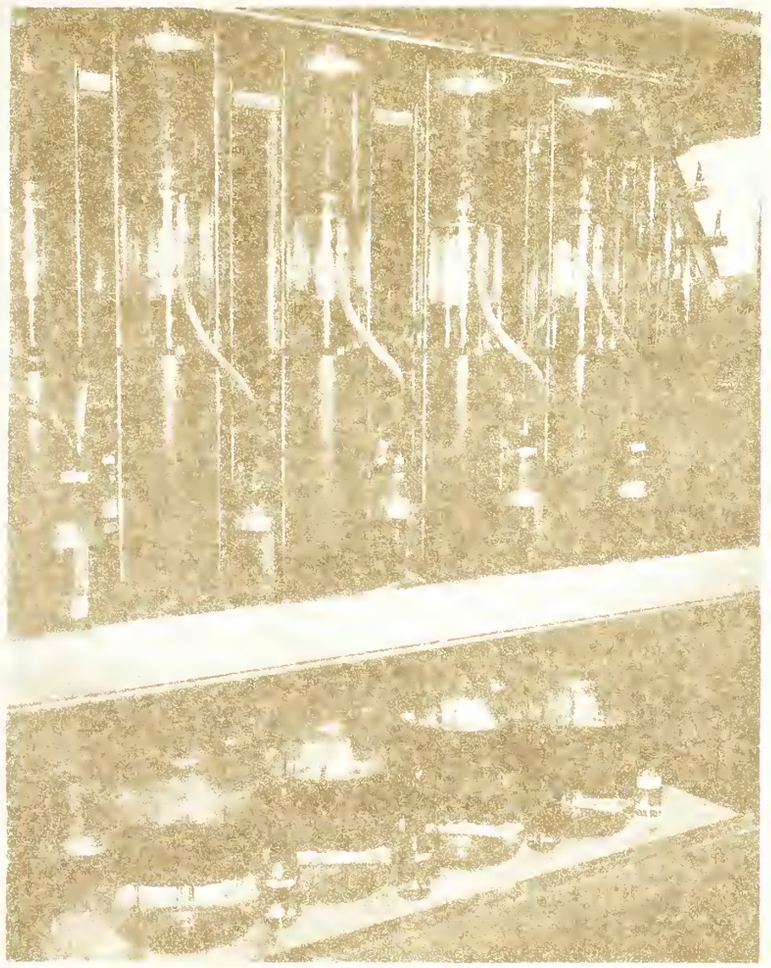
10. Any leak in the air lines or air cylinders does not expose the silage sample.

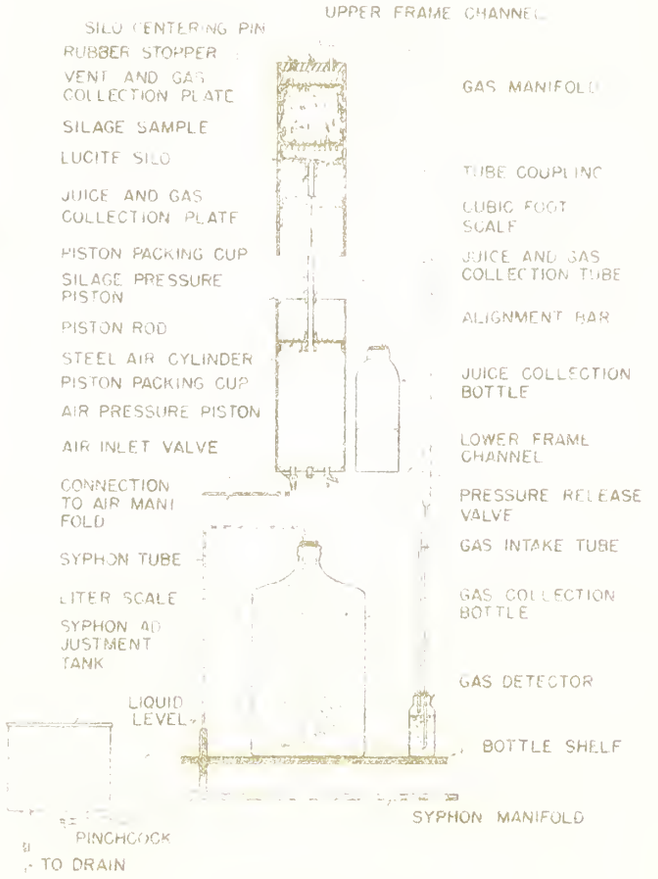
11. It is felt that any environmental condition known to exist in a large silo can be reproduced with this equipment. However, at present, controlled temperatures must be above that of the room in which the units are housed.

Performance of the equipment has been evaluated by loading all nine silos of a unit with identical material and producing silage in the same environment of temperature and pressure. Results of one such run

subjected to statistical analysis indicated the following probable errors at the 5% level: Total weight loss ± 2.3%; Volume change ± 2.3%; Total juice ± 2.7%; Total gas ± 11%; Total NO_2 ± 22%; Final moisture content ± 1% and final density ± 1%; acidity showed no measurable difference. Large error in total gas and total NO_2 appear to be due to methods of measurement. Improvements on methods have been made since this test was run.

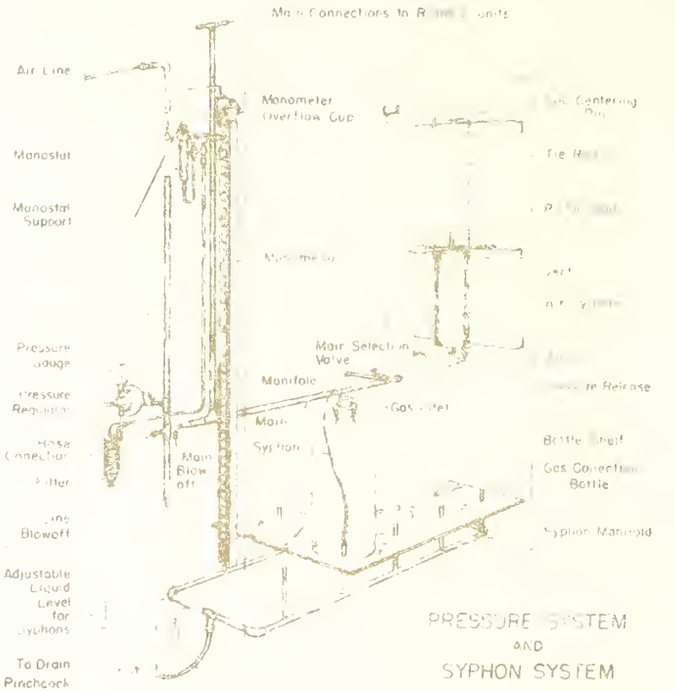
If further details are desired, they can be obtained from C. K. Otis, Minnesota Agricultural Experiment Station.





LABORATORY SILO

SCHEMATIC



PRESSURE SYSTEM
AND
SYPHON SYSTEM

S U M M A R Y O F S E C O N D S I L A G E C O N F E R E N C E

Held at Beltsville, Maryland on March 9-10, 1959

Part II

FACTORS AFFECTING CHEMICAL AND BACTERIOLOGICAL CHANGES

and

FACTORS AFFECTING SILAGE INTAKE AND PRODUCTIVE VALUE

FOREWORD

Part I of the Summary of the Second Silage Conference held at Beltsville, Maryland, March 9-10, 1959, deals primarily with procedures and techniques used in silage studies. Because of the revision and additions to the manual and the abstract material of the group discussion conducted the second day of the Conference, the size of the manual was increased considerably. Since it was not practical to combine the mimeograph material into one manual, it was summarized into two parts.

Part II of the Summary includes two phases of the group discussion (1) Factors Affecting Chemical and Bacteriological Changes in Silage Making; and (2) Factors Affecting Silage Intake and Production Value.

Since it was the desire of those present that some record be made of the two discussions, members of the Dairy Cattle Research Branch abstracted these discussions and they are included in summarized form. As the Branch personnel did not wish to assume responsibility for statements made by the various individuals, names and locations have been largely eliminated from these summaries. Certain parts of the summaries also include material of some workers who were not able to attend the Conference.

FACTORS AFFECTING CHEMICAL AND BACTERIOLOGICAL CHANGES

(a) Nitrogen fractions:

The topic on nitrogen fractions was introduced by reviewing the work of various English workers.

Barnett (3) has stated in his book on "Silage Fermentation" that protein degradation in silage is not doubt a result of plant enzymes--but that this action accounts only for the change (via intermediates) to amino acids. This matter becomes extremely complex when an attempt is made to differentiate between the plant and bacterial enzymes that function in the complex silage system. After the silo has been packed, aerobic respiration of the plant cells continue until all the oxygen has been exhausted. Along with these respiratory changes, protein breakdown may take place as a result of the activity of the proteolytic enzymes of the plant cells. If this is true, the composition of the substrate would be changing continuously as a result of the plant enzyme activity before and during the early stages of bacterial growth.

Several contradictory hypotheses have been advanced concerning the relative importance of plant cells and microorganisms in the ensilage process and various approaches have been made to the problem. Attempts have been made to study types and numbers of bacteria in forages and correlate them with chemical changes. Silage has been prepared from forages treated with antiseptics such as chloroform and toluene. It was hoped in this case to arrest action of bacteria, and, if possible, to attribute changes that occurred to the plant enzymes. Another method of attack has been to grow bacterial-free plants, pack them in tubes aseptically and study the changes that occur. In one such experiment, Mabbitt (7) showed the following results:

	Grass	Silage
Total Nitrogen (g.)	6.42	-
Amino-acids (ml. N/1 acid)	25.0	117.0
Volatile bases (ml. N/1 alkali)	1.5	18.0
Alcohol	0	0
Volatile acids	0	0
Lactic acids	0	0

The results are expressed in composition/100 g. dry matter. The extensive proteolysis that occurred is in agreement with the view that much of the protein breakdown in silage is due to the action of plant enzymes. The increase in volatile bases is contrary to the usual assumption that these bases arise from bacterial action. Evidence contrary to the views that volatile basis are formed by plant enzymes rather than bacterial enzymes has been presented by Kemble (6). He did show, however, that rapid proteolysis in the initial stages of ensiling was caused by plant enzymes. These enzymes did not produce substances which changed the pH from that of the fresh grass.

It is generally felt that extensive amino acid breakdown in silage is a result of certain sporeforming anaerobes. They are termed putrefactive bacteria when some of the by-products of the anaerobic respiration processes which occur are foul smelling. The products formed may include organic acids, alcohols, amines, mercaptans, H_2S, CO_2, and NH_3. Hydrogen sulfide, mercaptans, and such compounds as indole and skatol are responsible for foul odors. The lactic acid bacteria on the other hand (lactobacilli and streptococci) have very restricted abilities to break down amino acids and this lack of catabolic activity can be correlated with wide synthetic disabilities. These organisms concentrate certain amino acids in the free state within their internal environment prior to metabolism and it is possible that this acts as a form of compensation for loss of synthetic ability.

The fact that the lactic acid bacteria are fastidious and require these nitrogen fractions for growth and the effect these compounds may have in the nutrition of dairy cattle warrant more detail work in an attempt to understand their true role in the silage fermentation process.

The discussion on nitrogen fractions was opened with the presentation of a schematic representation of various nitrogen fractions and compounds which might occur in silage. Some methods for determining the different fractions were also discussed. It was pointed out that most of the work to date has been concerned with the volatile nitrogen fractions. This fraction may be the most important one from an organoleptic viewpoint; however, from an acceptability standpoint the animal may or may not agree. Some workers felt that most of the volatile nitrogen fraction was ammonia, others did not agree. It was pointed out that the amount of this fraction may be small and at the same time from an organoleptic standpoint give an indication of poor quality.

In measuring volatile bases, care should be exercised to prevent hydrolysis of amides. Low temperature steam distillation is satisfactory. The method presently used by one team of workers is based on the procedures published by Watson and Ferguson. Workers in New Zealand have found the Conway method to be satisfactory. The opinion was expressed that only a very small quantity of volatile amines are present in silages below pH5.2. No agreement was reached on this point, however, it was agreed that more work should be done to differentiate between the volatile amines and ammonia.

In some experiments, it has been shown that alanine and B-hydroxybutyric acid tend to be high in poor quality silages.

A question was raised as to whether there is any true protein in silage. No firm opinions were expressed.

The need to establish nitrogen patterns including the non-volatile fractions was stressed, both to establish relationship of these to quality and to nutritional value of forages.

One worker concluded from his experience and observations that the problems in studies on nitrogen fractions in silage are related primarily to weakness in analytical procedures. This is true, particularly in the quantitative measurements of non-protein nitrogen compounds. Further work should also be concentrated on the identification of compounds that are grouped together as the unidentified nitrogen containing fraction.

(b) NO_2 production:

Many farmers have at one time or another observed a brownish stream of gas flowing from the top of their silo. This material may accumulate in the chute of the silo and the forage may take on an orange to brown color. The silage gives strong color tests for nitrites and nitrates. This phenomenon has been observed primarily in corn silage; however, recent work has shown that any crop and any silo can produce nitrogen dioxide. Peterson et al. (9), Briggs (4), and Burris (5), have recently published work on the production of nitrogen oxides from silages. They feel that the widespread use of increasing concentrations of nitrogenous fertilizers has caused the increased hazards from NO_2. Burris (5) discussed the assimilation of nitrate from the soil by plants. He pointed out that plants do not accumulate high levels of ammonia, but they do accumulate nitrate in rather high concentrations. There are reports of plants having accumulated up to 10 percent of their dry weight as nitrate salts. Corn and oats are active accumulators of nitrate and the use of high levels of nitrogen fertilizers likely explains the large amount of nitrate currently encountered in many crop plants. The evidence that nitrate is really the source of the NO given off from silos was confirmed when N^{15}, enriched nitrate was supplied to fresh plant material in laboratory silos and mass spectrometer readings showed that the NO involved was enriched with N^{15}, the stable isotope of nitrogen.

Earlier work at Wisconsin by Peterson et al. (9) suggested that bacteria and not plant enzymes were responsible for the formation of the nitrogen oxides from silage. This deduction was based primarily on experiments where chloroform and toluene inhibited growth of bacteria, stopped fermentation as shown by CO_2 production and, consequently, no NO_2 was formed. Sterilization and re-inoculation of the forages resulted in the formation of both gases, but in greatly reduced amounts. The experiments all pointed to bacteria as the agent responsible for nitric oxide production. Later experiments by the Wisconsin workers (5) showed that the plant itself could convert nitrate to NO gas. Actually, it was shown that the formation of nitric oxide in silage could be accomplished by both plant enzymes and bacterial enzymes under anaerobic conditions. The proof of these reactions was accomplished by growing and ensiling aseptic plants and studying the evolved nitric oxide.

They showed further that full scale silos gave peak productions of nitric oxide 20-30 hours after ensiling. Production of NO was controlled by metabisulfite which blocked the enzymatic activity of the plant tissues and organisms in the silo.

Data was presented at the Conference on nitrogen dioxide in farm silages. Of 331 silages actually tested for the presence of nitrogen dioxide in the silo chute, 96 or about 30 percent actually showed positive results. The gas had been seen in 13 percent of the silos. It was produced from all crops tested including corn, oat, and grass silage. The content of N oxides often reach 4000 p.p.m. in the air of the silo chute. The safe tolerance is considered to be 10-25 p.p.m.

Experimentally, it could be demonstrated that NO_2 production was related to fertility and nitrate content of the forages ensiled. The nitrate content or level was considered to be the critical factor which determined the extent of oxide formation. It was pointed out that the apparent lack of relationship between fertility and NO_2 formation may be due to other factors affecting nitrate accumulation in the plant, such as dry weather. The production of N oxides appeared to be greater in high moisture crops. Gas formation reached a peak at about 36 hours after ensiling and stopped by the tenth day. There was some correlation between CO_2 and NO_2 production.

Sodium metabisulfite delayed N oxide evolution initially, but the rate of the gases increased after 2 or 3 days and exceeded the control by 20 percent by the end of the ensiling period (10 days).

It appears that any crop or any silo can be a potential source of nitrogen dioxide. Unfortunately, NO_2 cannot always be seen in dark rooms or dark chutes. It may not be concentrated enough to be seen, yet may injure humans.

The pattern of poisoning is usually of the pulmonary type characterized by coughing and shortness of breath. The illness may not be properly identified (usually pneumonia) and with a variety of trial and error treatments the patients may show improvement. However, they may later relapse and die (28-30 days).

The following precautions to avoid silage gas poisoning have been listed:

1. During silo filling, watch for irritating yellow or brown fumes in or near the silo. If you see such fumes, get away and stay away from the silo. The poisonous gases, NO_2 or one of the other oxides of nitrogen, are heavier than air and tend to settle downward and hover around the base of the silo.

2. Let no one enter the silo without first running the blower for 10-15 minutes to completely ventilate the silo, chute, and silo room. It is wise to do this during filling, too, and whenever anyone enters the silo during the first ten days after filling.

3. Leave the chute door open at the surface of the silage. This will prevent gases from accumulating at the top of the silage.

4. Provide extra ventilation at the base of the chute when the silo is attached to the barn. If you have a separate silage room, it is wise to make an outside door for this room and leave it open during and after filling so that gas can escape at the floor level.

5. Keep children and animals away from the silo area for 10 days after filling.

(c) Carbohydrate fractions:

Barnett (3) has suggested that too little attention has been given to the carbohydrate content of grass and silage because nitrogen has been easy to estimate and a value for protein may be obtained from the nitrogen figures. In many areas, interest has been centered upon the production of protein and until the last few years techniques and methods for detection of many carbohydrates in a mixture have been lacking.

Since large amounts of lactic and other acids are formed in fermentation and the amount of elementary hexoses and disaccharides present in the grass could hardly provide this quantity the question arises as to what reserve carbohydrate is used by the silage microorganism.

Work has been done by Salisbury et al. (10) to determine the relative amounts of acid produced by mixed cultures of silage organisms when offered various carbohydrates as sources of energy. Arabinose, glucose, sucrose, fructose and xylose resulted in the production of more acid than the other carbohydrates used. The least amount of acid was produced from starch, dextrin, inulin and rhamninose. It was brought out in this work that molasses was used as a preservative and that the organisms which developed most rapidly undoubtedly were the ones which could utilize the sugars of molasses most efficiently.

Various English workers have suggested that fructosan of grass may act as a reserve carbohydrate. No fructosan was found in clover and alfalfa and this may help explain the difficulty in ensiling these crops. Work by Waite and Boyd (11) on the changes of carbohydrates during the life cycle of four perennial grasses showed that there was little difference between the contents of glucose, fructose and sucrose throughout the growing season. There was a marked difference, however, in the way the fructosan content of rye grass varied with stage of growth compared with the other grasses. The fructosan content of the rye grass continued to rise steadily during the flower stage, whereas during the same stage of development in the other species, the fructosan content, after an initial rise, fell abruptly for a period of 2-3 weeks. They point out that the difference in behavior of the fructosan content of various grasses is important because in temperate climates fructosan is at times a major component of the grass dry matter, rising to as much as 20-25 percent.

Later work by Mackenzie and Wylam (8) who analyzed the carbohydrates in the leaf and stem of perennial rye-grass showed that there were individual variations in the amounts of glucose, fructose and sucrose present. However, no seasonal trends in these sugars were evident. The plant accumulated in the stem a reserve of fructosan which reached a maximum of 21 percent of the dry matter before it diminished in the autumn. They showed that the cell wall constituents followed the same pattern in both leaf and stem. Polygalactose remained less than 1% and polyarabinose increased from 1 to 3 percent. There was a gradual increase in cellulose, polyxylose and lignin as the plant matured.

Bailey (2) studied the carbohydrate content of red clover and found seven sugars and a natural glucoside when extracted in 80% ethanol. The sugars were tentatively identified as xylose, glucose, fructose, sucrose, a fructosyl-fructose, a fructosyl-glucose and an isomer of raffinose. Contrary to the work of others, the sugar pattern in red clover appeared to be different from that reported for grasses. The most interesting difference was the presence of free xylose in the clover extracts. Free pentoses do not appear to have been detected in grasses. They are generally considered to occur in plants only in the combined form.

Bailey (1) also studied the starch content of clovers and rye grass. Using a cold grinding technique followed by perchloric acid extraction, an iodine-staining polysaccharide was obtained. Paper chromatograms of acid and enzymatic hydrolysates showed that the polysaccharide was starch. Clover leaves were shown to contain 3 to 5.5 percent and rye-grass leaves 0.7 percent of starch on a dry weight basis.

There seemed to be various factors which affect the carbohydrate level in plants. Some of these factors are climatic conditions, stage of growth, fertilization, etc. There are also considerable differences among workers as to the proper approach in studying carbohydrate fractions. Some feel that greater effort should be exerted toward identifying the more simple sugars and others feel that more emphasis should be placed on the complex carbohydrate fractions.

It would appear, however, that as many of the fractions as possible should be examined whether they be simple or complex because of their energy value in fresh and preserved forage and their importance in silage making process.

The role of the carbohydrate fractions of plant material in the ensiling process was discussed. The discussion was opened with the presentation of an outline showing the principle carbohydrates that occur in green forage crops.

What are the principal carbohydrates occurring in green forage crops?

Referring to the Weende system of proximate analysis, we note the two familiar groups:

Nitrogen free extract and Crude fiber

In the N-free extract we find:

- lignin -- not a true carbohydrate
- hemicellulose which can be resolved into: pentosans - pentoses - largely xylose
and hexosans - hexoses - largely glucose
- pectin
- oligosaccharides - sucrose
- monosaccharides - fructosans - fructose - glucosans - glucose

In the crude fiber portion we find:

- cellulose - mostly X-cellulose, but may contain xylan and possibly some unchanged hemicellulose.

Which of these fractions are important in the ensiling process?

Probably not those in the crude fiber portion; cellulose is not readily attacked by silage microorganisms especially if other and more readily fermentable carbohydrates are present.

Turning to the N-free extract what do we find and which fractions are fermented:

- lignin, certainly not; it resists bacterial action rather completely.
- hemicellulose - rather doubtful.
- pectin - mostly uronic acids - also doubtful
- this leaves us with the oligosaccharides, the most important of which is sucrose, and the monosaccharides glucose and fructose.

The latter occurs as fructosan which is considered to be the reserve carbohydrate of grasses and it probably plays an important part in good ensilage.

It appears then that the fraction of the carbohydrates of grass which plays the important role in silage fermentation is that which contains the so-called readily fermentable carbohydrates, sucrose, glucose, and fructose, either as such or in the form of "osans," fructose or fructosan apparently being the most important.

Results from studies discussed below give support to the above statement. For several years, determinations were made as a routine procedure for total sugar (expressed as invert sugar) in the green forage used for ensiling. Results available from 130 lots of forage showed an average sugar content of 5.6 percent of the dry substance. Routine fodder analyses were also made on these forages. Comparison of the N-free extract and crude fiber content of the forage and the resultant silage of a considerable number of the lots showed the following average figures:

	% N.F.E.	% Fiber
Before ensiling	45.8	29.0
After ensiling	40.1	34.3
Difference	-5.7	+5.3

Note the close agreement between the sugar content of the green forage and the decrease in N.F.E. after ensiling (5.6 cf 5.7). Such agreement is probably fortuitous, but it strengthens the observation that the simple sugars probably are the principal substrate in silage fermentation.

Note also the increase in fiber; presumably this is only relative due to the decrease in N.F.E., but it is of the same general order whether viewed from an absolute - 5.7% cf 5.3% - or a relative basis, - 16.8% cf + 18.3%.

It should also be noted, however, that the decrease in N.F.E. is not by any means total loss; conversion of the sugars to short-chain fatty acids has taken place and these appear in part at least in an increased value for ether extract. Since most of them are readily soluble in water,undoubtedly some portion is lost in seepage run-off.

In the light of the above what are some of the problems involved in this phase of the ensiling process? A few are listed below. The order in which they appear does not indicate their relative importance which may vary with any particular set of circumstances. More work is need on the:

1. Quantitative estimation of the carbohydrates present in different forage species, with special emphasis on determination of those that are readily fermentable, notably fructose, glucose, and sucrose.

2. Correlation of the results obtained in (1) with quality in silage as indicated by pH, and percentages of the several acids, notably lactic and butyric acid. Such correlation should be made first on silages to which no supplements have been added, passing later to those that have been supplemented.

3. Ways and means of furnishing suitable substrates for the development of the desirable lactic acid fermentation, e.g.: Use of species high in readily fermentable carbohydrate; effect of weather conditions just previous to harvest on accumulation of sugars in plant tissue and effect of time of day of cutting.

4. Quantitative study of carbohydrates present in commonly used additives as an aid to formulating the suitable substrates mentioned under (3).

It was pointed out that there may be seasonal fluctuations in sugar content of plants. Results from a two year study showed that sugar content was highest about 10:00 AM in mid-May. The temperature did not exceed 85°F. In later experiments, the highest sugar content was observed about 2 PM in August.

A question was asked concerning the role of hemicellulose as a reserve carbohydrate. It was suggested that hemicellulose is not readily attacked by lactic acid bacteria, but other bacteria present might utilize it to some extent. Little, if any, work has been done to determine if lactic acid bacteria can use this fraction.

Various other materials might be considered as possible sources of organic acid in silage, e.g., amino acids and organic acids in the fresh plant tissue.

It was noted that organic acids in the plant tissue may reach a concentration of two percent of the dry matter (malic, oxalic and citric acids). These acids may be metabolized by the lactic acid bacteria into the acids found in silage. It was pointed out that the acids in the plant tissue disappears soon after ensiling.

Some workers have found rather high concentrations of alcohols in silage. One worker reported more methyl than ethyl alcohol in a very dark silage at the bottom of a silo. The material was about a year and a half old and also contained some fusel oil (higher alcohol). One worker reported that traces of alcohol were usually found in good quality silages. Some silages showed large amounts of propyl alcohol.

Work was presented to show that lack of carbohydrate was seldom the limiting factor in the production of organic acids in a natural silage fermentation. Since considerable amounts of carbohydrate remained after fermentation, it was concluded that the benefit obtained from carbohydrate rich additives, such as ground snap corn, was not attributable entirely to the increase in amounts of carbohydrate substrate. When added citrus pulp on ground snap corn was added, drastic changes in pH were observed.

It was the general opinion that more work should be done on the efficiency of production of organic acids.

It was suggested that the refractometer might be a useful instrument to determine if a forage additive is needed or not. Juice is squeezed out of the fresh plant material and the percent sugar read directly on the refractometer. It measures total solubles which may be largely sugars. No firm opinions were expressed on the possible use of the instrument.

A considerable amount of work has been done over the years on the chemical composition of forages. Many problems are still involved in attempts to accurately define or obtain various plant fractions. Enclosed is a diagram of some of the various plant fractions along with their appoximate percentages and solubility.

The figure shows the relationships between chemical composition and the various proximate analyses that might be applied to forages or silages. In addition to the Weende distinctions of Ether extract, crude fiber and nitrogen free extract, other types of extractions are included such as alcohol, boiling water and chlorite holocellulose. These latter procedures are part of an attempt at Beltsville to develop a more satisfactory analysis of forages, that would determine the principal constituents not by difference as in NFE. The goal is to arrive at a scheme which divides the constituents into categories of similar chemical composition and nutritive value such as lignin, holocellulose, water soluble carbohydrates, etc. It is also hoped to arrive at an index of chemical composition that will provide a more accurate estimation of digestibility. The dry matter scale in the figure is adjusted to approximately that of alfalfa hay. Silages will have a larger volatile loss than is indicated.

(d) Bacterial flora:

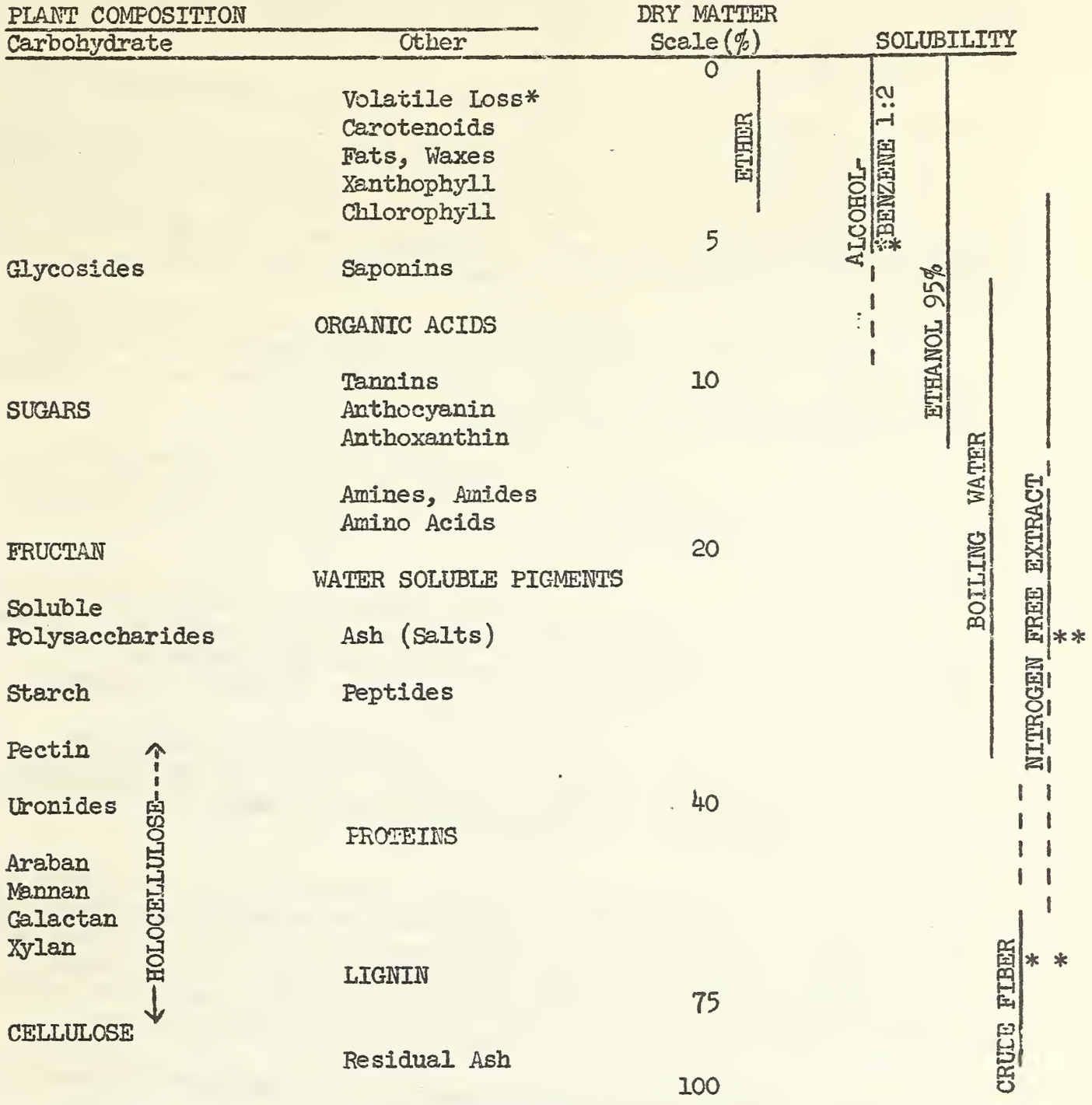
There are a variety of microorganisms that may be found on fresh plants and in silage. One group reported that the predominant organism they found on fresh plants was a facultative anaerobic species of Flavobacterium. This organism increased from 10^8 per g in late May to 10^{10} per g in early August. The Flavobacterium group on fresh forage did not multiply in the silage but persisted throughout the storage period in spite of the acid produced. The correlation between bacterial numbers on fresh grass and harvesting date indicated that the harvest date exerts control on the bacterial composition of the raw material from which silage is made.

Less than one percent of the bacteria on the fresh grass were lactobacilli. However, they increased in the silage to 10^9 per g soon after ensiling then dropped to about 10^5 per g after three weeks in the silo. A secondary fermentation occurred which reached a peak in four or five weeks then declined. This pattern was evident in both good and poor silages. The lactics on fresh and ensiled forages varied morphologically and on a fermentation basis. They were divided into two main types; one which fermented all of the test carbohydrates (simple and complex) and the ones that fermented only simple sugars. No differences were found in the lactic flora from the fresh plants and those found in primary and secondary fermentation.

The quality of the silages studied was determined primarily by the amount of packing they received. Tightly packed forages gave butyric and some propionic acids. Lactic and succinic acids disappeared. Well-preserved silages were optimally packed and loosely packed silages overheated and underwent an acetic acid fermentation.

Clostridium tyrobutyrium could be isolated immediately after ensiling. All of the 171 obligate anaerobes studied fermented lactate and none hydrolyzed gelatin. Volatile base was not associated with any particular

CHEMICAL COMPOSITION OF FORAGES



BROKEN LINES INDICATE PARTIAL SOLUBLIZATION

*Volatile loss consists of a wide variety of materials depending on the nature of the feed, eg. silage etc. Organic acids, amines, ammonia, terpene hydrocarbons, essential oils, aldehydes and other compounds contribute to this loss.

**Broken lines indicate partial solubilization of the constituents. Chlorite holocellulose preparations ordinarily contain some lignin and nitrogen.

organism but always occurred when the total hydrolyzable carbohydrate dropped below one percent of the fresh weight of the plant. High moisture crops tended to undergo the volatile base type spoilage because the carbohydrate was initially diluted.

A summary of the studies of another group of workers showed that:

(1) A succession of bacterial types has been observed to occur during the early stages in the fermentation of "good" quality alfalfa silages. In order of their appearance, these types are as follows: (a) Gram negative rods; (b) Gram positive chainforming cocci; (c) pediococci; (d) Gram positive paired rods; (e) Gram positive chain-forming rods.

(2) The succession of bacterial types during the fermentation of "poor to fair" quality silages appears to be characterized by an almost complete absence of the pediococci. In general, the poor quality silages have an extended period of Gram negative rod activity.

(3) A correlation between the bacterial types and the chemical changes which occur during fermenation has also been noted. The chemical changes measured were pH, organic acids and analysis of soluble carbohydrates.

(4) Comparison of the fermentation patterns of sodium metabisulfite silages with untreated controls showed no significant differences between the bacteriological patterns; however, the rate of disappearance of various sugar fractions was decreased under the influence of bisulfite.

In a detailed study of about 440 strains of bacteria isolated from silages, another group of workers showed that the genera Streptococcus, Leuconostoc, Pediococcus and Lactobacillus were responsible for most of the acid produced.

Later work on the early stages of silage fermentations showed that the plant flora consisted primarily of aerobic gram negative chromagenic and non-chromagenic rods along with fewer numbers of similar organisms that were facultative in their oxygen requirement. Most of these organisms disappeared by 48 hours after ensiling and none were observed at two weeks. The final pH of the organisms in glucose broth was seldom below 5.9.

The sequence change of the microorganisms during the first 48 hours of the fermentation process was usually as follows:

1. Gram negative low acid producing rods; (2) followed by acid producing cocci; and (3) which in turn were followed by the high acid producing rods.

It was agreed that more work should be done to clarify the relationship among the organisms which occur during the early phases of the fermentation process.

Some suggestions for further study were:

(1) The work on pediococci should be extended to a larger group of silages over two or three years to see if there is a significant correlation of the presence of these organisms with good quality.

(2) Studies on metabolic activities of the pediococci isolated from silage should help to better define their role in silage fermentation.

(3) Methods for aseptic production of or effective sterilization of samples of suitable forage would be of benefit in assessing the role of individual groups of microorganisms in preservation. Such methods could pave the way for pure culture studies and controlled mixtures of organisms.

(4) Determine if the present media used are adequate for isolating and studying lactic and bacteria, total anaerobes and spore-forming anaerobes.

(5) Attempt to more clearly define microorganisms on a physiological basis by using selective and/or enrichment media along with the application of "most probable numbers."

(6) Consider inoculation of fresh forage in the light of our knowledge of mutants.

(7) Attempt to identify possible inhibitory substances from fresh plants or bacteria that might retard growth of spore-forming anaerobes.

(8) Consider work to determine further if results obtained from miniature silos are comparable to farm size silos.

Oxygen--Aeration

It should be pointed out in the following discussion that differences in the oxygen content of silages is often produced by variations in such factors as density, length of cut, moisture content, speed of filling, etc. Results thus obtained are often confounded since it cannot be known which of the variables is primarily responsible. The introduction of known amounts of oxygen (aeration) after the silo is filled is one method of avoiding such complications.

Data were presented that showed when forage was aerated for 8 hours after ensiling that the peak temperature, percent ammonia nitrogen and percent butyric acid were higher in the silages as compared to non-aerated ones. High temperature persisted for a longer time in the aerated silages and oxygen was not present in detectable amounts shortly after aeration was stopped.

Data were also presented on successive layers of spoiled corn silage (separated by fiber glass mesh) which indicated that the proximity of spoilage to the surface had a major influence on the extent of gaseous losses occurring in the spoiled material. Gaseous losses ranging from 80 to 30 percent of the stored dry matter were observed in the spoiled layers, the greater losses occurring near the surface and smaller losses in material adjacent to good silage. These observations indicated that air infiltration is progressive in nature and that invisible gaseous losses may account for a major portion of the losses due to exposure.

Other work was presented which showed diagrams of density, pressure, moisture and acidity values in silage. The density pattern was obtained by core sampling about three months after filling in a farm size silo. The silo was filled with a mechanical distributor that threw the green material to the walls building up a funnel-shaped pile low at the center. The center was filled by the plant material falling to the center as the silos filled up. The acidity was determined by use of a pH indicator on the core samples. It was interesting to note the high pH values (4.6-6.4) around the chute doors and through a central core. Even though at the time the samples were taken the density was fairly high in the central regions, the pH values indicated a less favorable fermentation taking place as compared to other areas in the forage mass. It was suggested that oxygen had not been pressed out as quickly in this central region sampled as in other areas of the silage and that some oxygen infiltration occurred around the chute doors.

These experiments indicate the difficulty of separating oxygen effects from those of density, moisture and other factors which may affect exclusion or infiltration of oxygen into silage.

Length of Cut (Bruising)

This discussion was opened by briefly reviewing some British work and then pointing out the difficulty of evaluating this type of work since there is no true measure of the extent of bruising.

One group reported on two distinct physical treatments of plants. One consisted of chopping at 3" lengths, the other chopped at 5/16" length followed by passage through a Silerator (British harvesting machine designed to cause considerable bruising to plant tissue). The materials were then compared in a preliminary manner in sealed 4' x 8' silos.

The bruised forage was characterized by a more stable sugar content after storage in the silo and a very rapid drop in pH to 4.0. The sugar content of the non-bruised material declined more rapidly and after ensiling was characterized by pH values 4.5 to 5.0

Although greater density was attained in the bruised forage, other work at the same laboratory has shown that the same general effects are obtained from these treatments when density is the same. Thus, it was felt that the effect of the bruising was direct. The mechanisms whereby these differences are obtained are not clear. However, it was noted

in quart jar silos that the negative pressure established soon after bruising persisted much longer in the bruised material. This indicated a fundamental difference in biochemical activity.

Another investigator also reported work where a silerator had been used. The unchopped grass that was lacerated had a final pH of 4.2. A similar long chop grass that had not been lacerated had a final pH of 4.8. Laceration lowered losses and was more easily compacted. The overall results, however, were not as clear cut as expected.

Other workers using plastic bags and long and short cut forage (lacerated) under 3 pounds pressure found no differences in silages produced. The concluded that laceration had little effect on the final quality of the silages and that compactness or consolidation was more important.

Moisture

It was pointed out that moisture was a problem in several ways in silage making. It was suggested that a good drainage system in the bottom of the silo helps to prevent the juice from rising in the silo and the leaking of joints between staves often serve as safety valves relieving hydrostatic pressure. However, a good bottom drain may not be enough to eliminate waterlogging since density layers may increase enough in the bottom layers to stop percolation. It may also be true that juices build up in less dense areas of the silo causing low acid pockets rather than entrapment of air as was suggested earlier.

Some consideration was given to the prevention of plasmolysis in silage. It is known that it takes a great deal of pressure to remove moisture from fresh cut material, but very little after the same material has been confined for 24 hours or more. It was suggested by workers that KNO_3 added to green plant material might check the flow juice.

Top Covers

Various workers commented on the use of polyethylene and vinyl covers for stacks. Rodents caused considerable loss with polyethylene covers. This trouble was not experienced with vinyl, although vinyl tended to whip more.

It was noted that mold was a problem with polyethylene covers up to 10 mil in thickness but none was found with 6 mil vinyl.

The opinion was expressed that the major value of covers lie in preventing oxygen infiltration but that oxygen can pass through any cover depending on the type of plastic used. Thus, oxygen infiltration into plastic sealed silages may be an undesirable variable in experiments where air exclusion is intended to be equal. Slow rates of infiltration would be undetected since no visible spoilage would result.

Scoring of Silage Samples

During the Silage Conference (March 9-10, 1959) samples of forage were judged by the participants in an attempt to evaluate the revised score-card for grass silage as given in Part I. Forty participants evaluated duplicate samples of nine different silages. The organoleptic evaluation was based on color and odor of the silages. The descriptions of the forages are given below:

Sample Nos.	Type of Forage	Type of Silo	pH
1 12	Orchardgrass, 1st cut Direct-cut	Bunker Sealed	5.19
2 15	Alfalfa, 1st cut Direct-cut	Harvestore	4.51
3 18	Orchardgrass, 1st cut Direct-cut	Bunker Not-sealed	5.14
4 10	Haylage, 1st cut	Harvestore	4.90
5 11	Alfalfa, wilted	Concrete Stave	5.18
6 16	Alfalfa-grass mixture 3rd cut, direct-cut	4'x 8' Experimental	4.97
7 17	Alfalfa-grass mixture 3rd cut, lacerated	4'x 8' Experimental	3.98
8 14	Alfalfa, lacerated	Concrete Stave	4.45
9 13	Alfalfa, direct-cut	Concrete Stave	4.92

Since the samples were duplicates, this afforded the opportunity to check the individual participants ability to reproduce his score.

Table 1 gives the samples and scores, the average deviation or replication and the range of duplication. The average deviation from replication was $\pm$ 6.99 between the samples. The range of deviation was from $\pm$ 0-40.

Twenty participants (50 percent) had 5 or more samples scored within five points on duplicate samples.

There was close agreement on the top quality samples which would indicate that the relative ranking of silages can be accomplished.

It appears that organoleptic evaluation of silage has merit. However, for an individual to become competent in this field of endeavor he must have some background of training.

GRASS SILAGE EVALUATION

Participant Number	Samples and Scores									Ave. difference on replication	Number of Samples 5 or less	Range of difference
	1	2	3	4	5	6	7	8	9			
	12	15	18	10	11	16	17	14	13			
1	45	54	44	53	50	49	56	50	48			
	46	54	46	55	49	50	56	50	51			
	1	-	2	2	1	1	-	-	3	1.1	9	0-3
2	39	54	41	51	43	42	58	53	49			
	40	57	38	52	40	38	57	51	45			
	1	3	3	1	3	4	1	2	4	2.4	9	1-4
3	36	40	23	42	42	21	56	42	44			
	36	45	20	42	35	20	56	42	38			
	-	5	3	-	7	1	-	-	6	2.4	7	0-7
4	28	25	28	13	25	25	50	26	26			
	20	23	33	13	20	23	50	26	25			
	8	2	5	-	5	2	-	-	1	2.6	8	0-8
5	20	48	22	27	32	20	52	32	37			
	20	55	20	32	40	20	52	32	41			
	-	7	2	5	8	-	-	-	4	2.9	7	0-8
6	39	48	38	35	39	42	54	40	35			
	38	51	40	44	35	35	54	38	35			
	1	3	2	9	4	7	-	2	-	3.1	7	0-9
7	46	54	45	42	52	40	55	49	42			
	41	50	50	42	52	46	55	52	50			
	5	4	5	-	-	6	-	3	8	3.4	7	0-8

Participant Number	Samples and Scores 1	2	3	4	5	6	7	8	9	Ave. difference on replication	Number of Samples 5 or less	Range of difference
	12	15	18	10	11	16	17	14	13			
8	42	43	33	26	35	24	48	34	38			
	32	39	35	28	28	27	50	28	39			
	10	4	2	2	7	3	2	6	1	4.1	6	1-10
9	38	50	36	42	27	39	53	35	33			
	34	50	46	48	32	42	53	42	36			
	4	-	10	6	5	3	-	7	3	4.2	6	0-10
10	30	52	30	40	44	36	42	40	40			
	36	52	36	32	36	32	44	40	44			
	6	-	6	8	8	4	2	-	4	4.2	5	0-8
11	41	46	46	37	39	42	50	50	53			
	32	42	43	35	48	41	54	49	47			
	9	4	3	2	9	1	4	1	6	4.3	6	1-9
12	40	53	35	43	25	30	57	40	46			
	27	51	45	40	34	32	56	35	47			
	13	2	10	3	9	2	1	5	1	5.1	6	1-13
13	33	48	46	23	29	18	54	31	39			
	22	49	34	24	30	29	54	32	30			
	11	1	12	1	1	11	-	1	9	5.2	5	0-12
14	30	40	40	20	30	40	50	25	37			
	40	40	40	30	30	40	50	35	45			
	10	-	-	10	-	-	10	10	8	5.3	4	0-10
15	35	41	34	29	45	21	42	32	32			
	30	50	38	42	35	37	43	30	34			
	5	9	4	13	10	16	1	2	2	6.9	5	1-16

Participant Number	Samples and Scores 1	2	3	4	5	6	7	8	9	Ave. difference on replication	Number of Samples 5 or less	Range of difference
	12	15	18	10	11	16	17	14	13			
16	36	40	45	16	28	35	53	22	45			
	30	48	38	27	28	17	42	20	43			
	6	8	7	11	-	18	11	2	2	7.2	3	0-18
17	27	30	42	20	30	36	53	40	30			
	25	52	30	20	35	20	52	40	38			
	2	22	12	-	5	16	1	-	8	7.3	5	0-22
18	38	44	35	28	35	26	54	38	34			
	29	55	39	49	33	30	49	43	39			
	9	11	4	21	2	4	5	5	5	7.3	6	2-21
19	38	48	44	43	41	30	55	47	55			
	24	50	37	34	33	43	49	52	58			
	14	2	7	9	8	13	6	5	3	7.4	3	3-14
20	27	53	50	20	30	53	45	45	40			
	45	50	45	20	35	52	40	35	60			
	18	3	5	-	5	1	5	10	20	7.4	6	0-20
21	42	47	39	24	42	40	49	33	35			
	33	54	43	35	42	50	38	45	40			
	9	7	4	11	-	10	11	12	5	7.7	3	0-12
22	40	26	26	18	28	20	58	40	48			
	25	32	20	20	20	15	58	12	48			
	15	6	6	2	8	5	-	28	-	7.7	4	0-28
23	35	50	40	30	35	35	50	35	40			
	25	55	25	40	45	20	50	35	35			
	10	5	15	10	10	15	-	-	5	7.7	4	0-15

Participant Number	Samples and Scores									Ave. difference on replication	Number of Samples 5 or less	Range of difference
	1	2	3	4	5	6	7	8	9			
	12	15	18	10	11	16	17	14	13			
24	40	47	50	30	41	28	44	56	44			
	52	54	32	30	34	20	46	56	56			
	12	7	18	-	7	8	2	-	15	7.7	3	0-18
25	40	50	56	25	28	39	60	38	32			
	32	42	50	20	36	42	60	20	46			
	8	8	6	5	8	3	-	18	14	7.8	3	0-18
26	34	41	49	20	24	51	49	50	46			
	49	50	34	20	24	50	52	26	43			
	15	9	15	-	-	1	3	24	3	7.8	5	0-24
27	35	45	30	40	40	30	55	30	40			
	15	50	30	30	35	30	50	55	40			
	20	5	-	10	5	-	5	25	-	7.8	6	0-25
28	28	46	27	34	34	22	60	37	43			
	50	60	18	41	41	21	58	35	51			
	22	14	9	7	7	1	2	2	8	8.0	3	1-22
29	28	50	32	31	25	30	55	55	44			
	41	55	34	40	44	17	56	49	51			
	13	5	2	9	19	13	1	6	7	8.3	3	0-19
30	27	49	25	38	35	13	55	51	40			
	38	42	38	36	52	25	42	51	38			
	11	7	13	2	17	12	13	-	2	8.6	3	0-17
31	43	51	32	50	46	23	54	51	46			
	26	51	41	21	41	28	55	44	41			
	17	-	9	29	5	5	1	7	5	8.7	5	0-29

Participant Number	Samples and scores 1	2	3	4	5	6	7	8	9	Ave. difference on replication	Number of Samples 5 or less	Range of difference
	12	15	18	10	11	16	17	14	13			
32	30	52	46	50	42	45	50	46	52			
	53	46	47	35	45	51	56	28	50			
	23	6	1	15	3	6	6	18	2	8.9	3	1-23
33	50	56	30	41	33	38	36	41	41			
	31	51	23	46	44	40	52	46	52			
	19	5	7	5	11	2	16	5	11	9.0	4	2-19
34	32	50	25	28	42	30	53	50	35			
	25	57	30	25	22	35	57	26	42			
	7	7	5	3	20	5	4	24	7	9.1	4	3-24
35	32	54	32	31	40	21	52	31	32			
	50	52	31	45	31	30	60	20	45			
	18	2	1	14	9	9	8	11	13	9.4	2	1-14
36	32	56	47	53	29	53	55	54	51			
	45	45	50	39	36	32	40	47	50			
	13	11	3	14	7	21	15	7	1	10.2	2	1-21
37	37	55	24	20	37	29	58	41	46			
	14	50	49	30	26	30	58	33	35			
	23	5	25	10	11	1	-	8	11	10.4	3	0-25
38	5	55	50	15	10	26	60	50	35			
	30	60	35	30	40	40	60	45	40			
	25	5	15	15	30	14	-	5	5	12.7	4	0-30
39	30	25	10	10	25	40	40	15	15			
	10	15	50	20	10	15	40	10	15			
	20	10	40	10	15	25	-	5	-	13.9	3	0-40
40	20	20	25	13	28	12	50	25	17			
	45	33	55	12	15	33	35	15	15			
	25	13	30	1	13	21	15	10	2	14.4	2	0-30

FACTORS AFFECTING SILAGE INTAKE AND PRODUCTIVE VALUE

Because of time limitation, the discussion of this topic was somewhat limited. It is apparent from the reports in the literature that dairy cattle do not consume as much dry matter in the form of silage as they will from good quality hay made from the same field. This effect is more marked with growing heifers than with milking cows. Since the cause of this effect is not apparent, the purpose of the discussion was to point out the pertinent factors involved, to discuss possible reasons for the effect, and to obtain suggestions for studies to determine the cause of the effect.

It was reported that replacing silage with hay in the ration of dairy cows resulted in greater dry matter consumption and higher milk yields. The magnitude of these differences was directly related to the extent to which hay replaced silage. Heifers consumed more alfalfa dry matter when it was fed as hay than when fed as wilted silage and least dry matter was consumed from lacerated high moisture silage. Consumption of hay dry matter was not depressed by the addition of water or HCl. However, the addition of ground hay to high moisture silage, at the time of feeding, did increase dry matter consumption. The work indicated a generally lower dry matter consumption from silage, particularly high moisture silage as compared to hay. Lower rates of animal production (milk or growth) have accompanied the lower dry matter intake.

A researcher from another location reported difficulties encountered when alfalfa hay was replaced by alfalfa silage in the rations of dairy heifers on a limited milk grain regime. Substitution of wilted alfalfa silage for the hay resulted in sub-normal rates of gain, dry matter intake, and weight at calving time. Supplementing the wilted alfalfa silage with alfalfa hay (0.5 or 1.0 lb. per cwt.) or grain (2 lb/day) improved performance, but it was still below that obtained on hay only. A ration of wilted alfalfa silage and corn silage produced heifers that were inferior to those on alfalfa silage only. Thus, the corn grain in the corn silage did not overcome the difficulty. Further work on material cut simultaneously from alternate areas of the same field have confirmed and extended the earlier results.

Increased consumption of wilted alfalfa silage by growing heifers was not obtained when any of the following were added to the silage just before feeding, (1) molasses, (2) flavor compound (Aroma-pep), (3) limestone, (4) potassium orotate and methionine, (5) special appetizer booster pellets and (6) 2 lb. grain per day.

Changing the water content of silage by drying it to hay consistency did not change the voluntary intake or gain of heifers fed this material compared to that of heifers receiving the silage in its original form.

Addition of water to hay and soaking for 4-12 hours did not result in an absolute or comparative reduction in dry matter consumption. Soaking the hay in silo effluent has reduced intake somewhat.

Addition of the silo effluent directly to the rumen through a fistula of heifers fed hay has reduced the hay dry matter consumed. This effect was noted with fresh and/or mature alfalfa or corn silo effluent. This effect was not apparent when 340 gm. of lactic and 130 gm. of acetic acid (neutralized to pH 5.5) was given daily through a rumen fistula.

Several experiments have indicated that a positive relationship existed between the dry matter content of the silage and the amount of dry matter that heifers would consume. However, the dry matter content of silage was decreased at feeding time by the addition of water there was no concomittant decrease in dry matter intake.

These trials indicate that wilted alfalfa silages have limitations as a feed for growing dairy herd replacements.

Most alfalfa silages contain some factor(s) that reduces the gain and voluntary intake of heifers. The satiety value of silage is apparently higher than that of hay. Under ordinary conditions, this effect is positively related to the water content of the silage but it is not the water content per se. This effect is probably related to the extent or type of fermentation that occurs in the silo. These trials have not provided positive information on reasons for the reduced voluntary intake when silage is fed to growing dairy heifers.

It was reported from another state that the dry matter intake of dairy cows fed silage made from a clover-timothy crop (75:25) was related to the method of preparation and date of cutting. Of particular interest were the experiments in which early cut hay and early cut wilted silage have been compared as the sole roughage for dairy cows. The silage fed cows consumed significantly less dry matter but produced significantly more milk and liveweight gains. These continuous feeding trials have indicated that energy from these silages has been more efficiently utilized and/or contains some lactogenic factor not contained in the hay. It was suggested that this question could be resolved by net energy studies.

A representative from one state commented that his group had observed a consistently higher dry matter consumption by milking cows from fresh cut second cutting silage than from first cutting silage.

A general discussion of factors affecting intake and productive value followed. It was generally concluded that the water content per se of grass or legume silage was not the factor involved in the acceptability of the silage by the animal. However, it was generally conceded that the water content of the crop at the time of ensiling does have considerable to do with acceptability. Therefore, the factor or factors involved must develop during the fermentation process which takes place following the ensiling of the crop. Whether the changes produced are due to the plant enzyme or bacterial enzyme systems is not clear.

There is some suggestion that the effect may not be due to the organic acids produced during the fermentation process. However, the data on this point are fragmentary and should be pursued further. It was further suggested that the effect might be due to some protein or nitrogen fermentation products. It would seem quite worthwhile to make a rather intensive study of the N-fractions since ensiling results in marked changes in their partition.

In further discussion, it was concluded that it is not definitely known whether the acceptability of grass or legume silage is due to reduced palatability per se, to the lack of some factor in the silage, or to the development of some nutrient in the silage which the animal might have difficulty in metabolizing. There is some indication that the effect might be due to the latter cause since the introduction of silage effluent through a rumen fistula caused some decrease in the consumption of alfalfa hay. Whatever the cause of the poor acceptability of silage, it was thought that the effect was much greater in growing heifers and dry cows than in lactating cows.

It was brought out that lowered dry matter consumption of silage might be a voluntary refusal by the animal due to palatability, or an involuntary response to some metabolic disturbance resulting from high silage intakes. It was suggested that errors in silage dry matter content determinations could account for a part of the lower consumption observed but that the expected error would be much too small to account for the differences observed.

As an explanation for the apparently more efficient dry matter utilization observed in milking cows by one group for some reports, it was proposed that the formation of lactic acid in the silo represented an economy in the work of digestion from the animal standpoint. In other words, the energy loss due to the heat increment in the digestion and metabolism of carbohydrates to form organic acids in the animal has already taken place in the silo. However, this does not explain why all high lactic acid silages do not follow the same efficiency pattern.

Since the feeding value of silage appears to improve as its characteristics approach that of hay, it was suggested by some that efforts should be made experimentally to suppress the fermentation of the silage as much as possible. The possibilities of accomplishing this through chemical additions or by partial drying was briefly discussed. Such a procedure might throw some light on the problem.

REFERENCES:

(1) Bailey, R. W. 1958. J. Sci. Food Agric., 9, 743-747.

(2) Bailey, R. W. 1958. J. Sci. Food Agric. 9, 748-753.

(3) Barnett, A. G. 1954. Silage Fermentation. Academic Press, Inc. New York.

(4) Briggs, Rodney A. 1959. Minnesota Farm and Home Science XVI, 18-19.

(5) Burris, R. H. 1958. Second Wisconsin Silage Conference Proceedings. Wisconsin Center, Madison, Wisconsin.

(6) Kemble, A. R. 1956. J. Sci. Food Agric. 2, 125-130.

(7) Mabbitt, L. A. 1951. Proc. Soc. Appl. Bacteriol. 14, 147-150.

(8) Mackenzie, D. J. and Wylam, C. B. 1957. J. Sci. Food Agric. 8, 38-45.

(9) Peterson, William H., Burris, Robert H., Sant. Rameschchandra, and Little, Henry N. 1959. Agric. and Food Chem. 6, 121-126.

(10) Salisbury, R. L., Mather, R. E. and Bender, C. B. 1949. J. Dairy Sci. 32, 901-906.

(11) Waite, R. and Boyd, J. 1953. J. Sci. Food Agric. 4, 197-204.